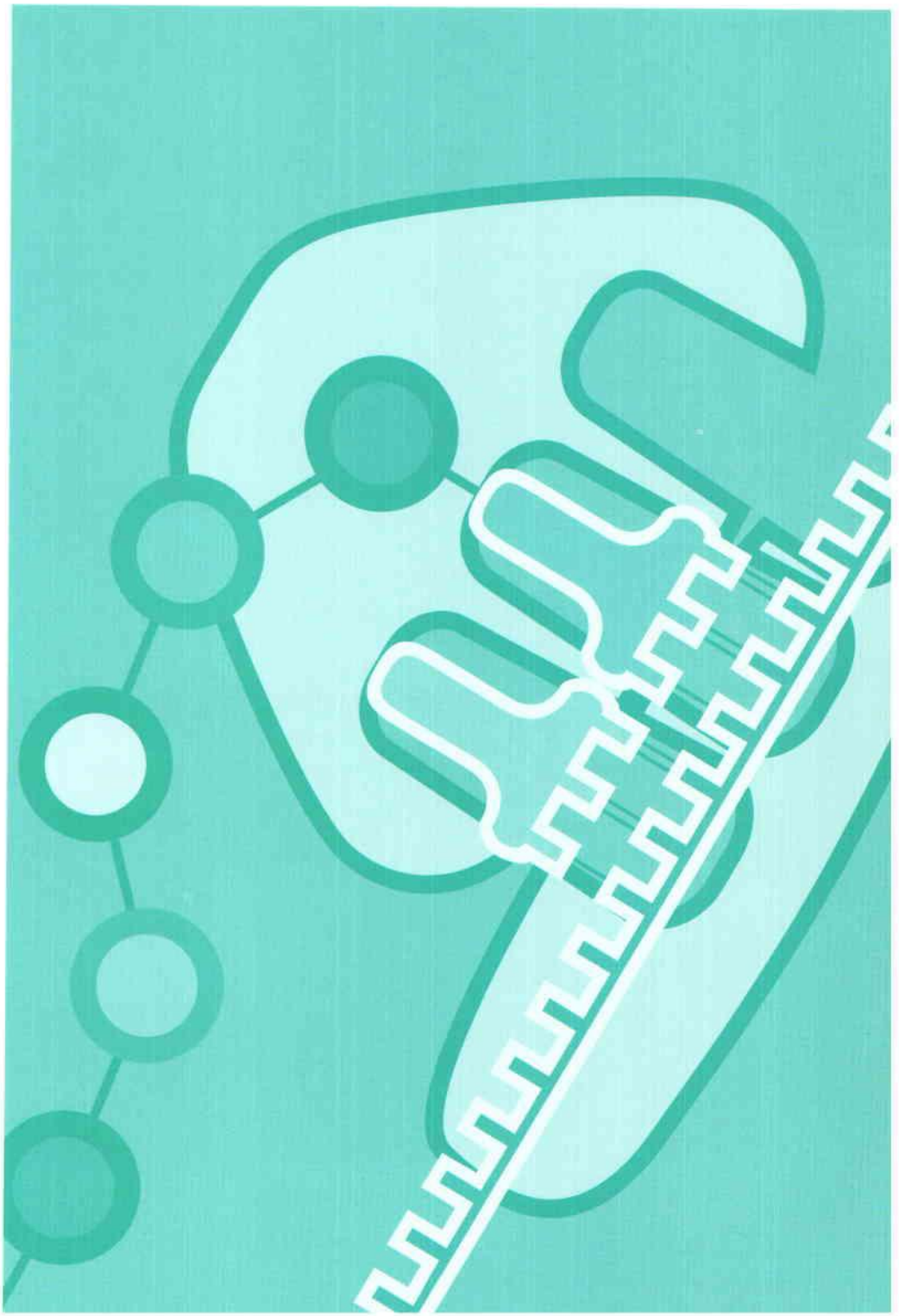
INTRODUCTION TO THE CELL

Part I
Chapters

1 Cells and Genomes

2 Cell Chemistry and Biosynthesis

3 Proteins



1 Cells and Genomes

The surface of our planet is populated by living things—curious, intricately organized chemical factories that take in matter from their surroundings and use these raw materials to generate copies of themselves. The living organisms appear extraordinarily diverse. What could be more different than a tiger and a piece of seaweed, or a bacterium and a tree? Yet our ancestors, knowing nothing of cells or DNA, saw that all these things had something in common. They called that something "life," marveled at it, struggled to define it, and despaired of explaining what it was or how it worked in terms that relate to nonliving matter.

The discoveries of the past century have not diminished the marvel—quite the contrary. But they have lifted away the mystery as to the nature of life. We can now see that all living things are made of cells, and that these units of living matter all share the same machinery for their most basic functions. Living things, though infinitely varied when viewed from the outside, are fundamentally similar inside. The whole of biology is a counterpoint between the two themes: astonishing variety in individual particulars; astonishing constancy in fundamental mechanisms. In this first chapter we begin by outlining the universal features common to all life on our planet. We then survey, briefly, the diversity of cells. And we see how, thanks to the common code in which the specifications for all living organisms are written, it is possible to read, measure, and decipher these specifications to achieve a coherent understanding of all the forms of life, from the smallest to the greatest.

In This Chapter

THE UNIVERSAL FEATURES OF CELLS ON EARTH 1

THE DIVERSITY OF GENOMES AND THE TREE OF LIFE 11

GENETIC INFORMATION IN EUCARYOTES 26

THE UNIVERSAL FEATURES OF CELLS ON EARTH

It is estimated that there are more than 10 million—perhaps 100 million—living species on Earth today. Each species is different, and each reproduces itself faithfully, yielding progeny that belong to the same species: the parent organism hands down information specifying, in extraordinary detail, the characteristics that the offspring shall have. This phenomenon of *heredity* is central to the definition of life: it distinguishes life from other processes, such as the growth of a crystal, or the burning of a candle, or the formation of waves on water, in which orderly structures are generated but without the same type of link between the peculiarities of parents and the peculiarities of offspring. Like the candle flame, the living organism consumes free energy to create and maintain its organization; but the free energy drives a hugely complex system of chemical processes that is specified by the hereditary information.

Most living organisms are single cells; others, such as ourselves, are vast multicellular cities in which groups of cells perform specialized functions and are linked by intricate systems of communication. But in all cases, whether we discuss the solitary bacterium or the aggregate of more than 10^{13} cells that form a human body, the whole organism has been generated by cell divisions from a single cell. The single cell, therefore, is the vehicle for the hereditary information that defines the species (**Figure 1–1**). And specified by this information, the cell includes the machinery to gather raw materials from the environment, and to construct out of them a new cell in its own image, complete with a new copy of the hereditary information. Nothing less than a cell has this capability.

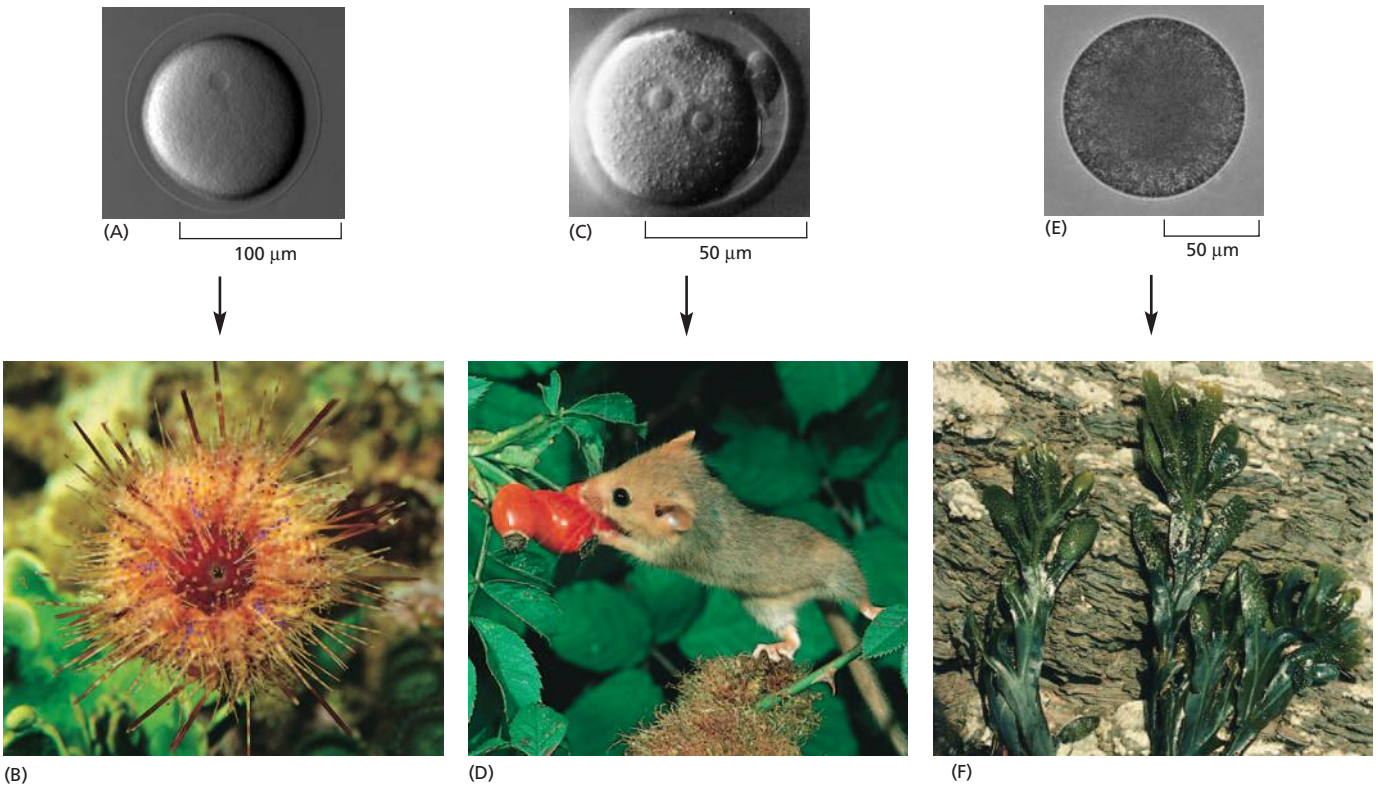


Figure 1–1 The hereditary information in the fertilized egg cell determines the nature of the whole multicellular organism. (A and B) A sea urchin egg gives rise to a sea urchin. (C and D) A mouse egg gives rise to a mouse. (E and F) An egg of the seaweed *Fucus* gives rise to a *Fucus* seaweed. (A, courtesy of David McClay; B, courtesy of M. Gibbs, Oxford Scientific Films; C, courtesy of Patricia Calarco, from G. Martin, *Science* 209:768–776, 1980. With permission from AAAS; D, courtesy of O. Newman, Oxford Scientific Films; E and F, courtesy of Colin Brownlee.)

All Cells Store Their Hereditary Information in the Same Linear Chemical Code (DNA)

Computers have made us familiar with the concept of information as a measurable quantity—a million bytes (to record a few hundred pages of text or an image from a digital camera), 600 million for the music on a CD, and so on. They have also made us well aware that the same information can be recorded in many different physical forms. As the computer world has evolved, the discs and tapes that we used 10 years ago for our electronic archives have become unreadable on present-day machines. Living cells, like computers, deal in information, and it is estimated that they have been evolving and diversifying for over 3.5 billion years. It is scarcely to be expected that they should all store their information in the same form, or that the archives of one type of cell should be readable by the information-handling machinery of another. And yet it is so. All living cells on Earth, without any known exception, store their hereditary information in the form of double-stranded molecules of DNA—long unbranched paired polymer chains, formed always of the same four types of monomers. These monomers have nicknames drawn from a four-letter alphabet—A, T, C, G—and they are strung together in a long linear sequence that encodes the genetic information, just as the sequence of 1s and 0s encodes the information in a computer file. We can take a piece of DNA from a human cell and insert it into a bacterium, or a piece of bacterial DNA and insert it into a human cell, and the information will be successfully read, interpreted, and copied. Using chemical methods, scientists can read out the complete sequence of monomers in any DNA molecule—extending for millions of nucleotides—and thereby decipher the hereditary information that each organism contains.

All Cells Replicate Their Hereditary Information by Templated Polymerization

The mechanisms that make life possible depend on the structure of the double-stranded DNA molecule. Each monomer in a single DNA strand—that is, each **nucleotide**—consists of two parts: a sugar (deoxyribose) with a phosphate group attached to it, and a *base*, which may be either adenine (A), guanine (G), cytosine (C) or thymine (T) (**Figure 1–2**). Each sugar is linked to the next via the phosphate group, creating a polymer chain composed of a repetitive sugar-phosphate backbone with a series of bases protruding from it. The DNA polymer is extended by adding monomers at one end. For a single isolated strand, these can, in principle, be added in any order, because each one links to the next in the same way, through the part of the molecule that is the same for all of them. In the living cell, however, DNA is not synthesized as a free strand in isolation, but on a template formed by a preexisting DNA strand. The bases protruding from the existing strand bind to bases of the strand being synthesized, according to a strict rule defined by the complementary structures of the bases: A binds to T, and C binds to G. This base-pairing holds fresh monomers in place and thereby controls the selection of which one of the four monomers shall be added to the growing strand next. In this way, a double-stranded structure is created, consisting of two exactly complementary sequences of As, Cs, Ts, and Gs. The two strands twist around each other, forming a double helix (Figure 1–2E).

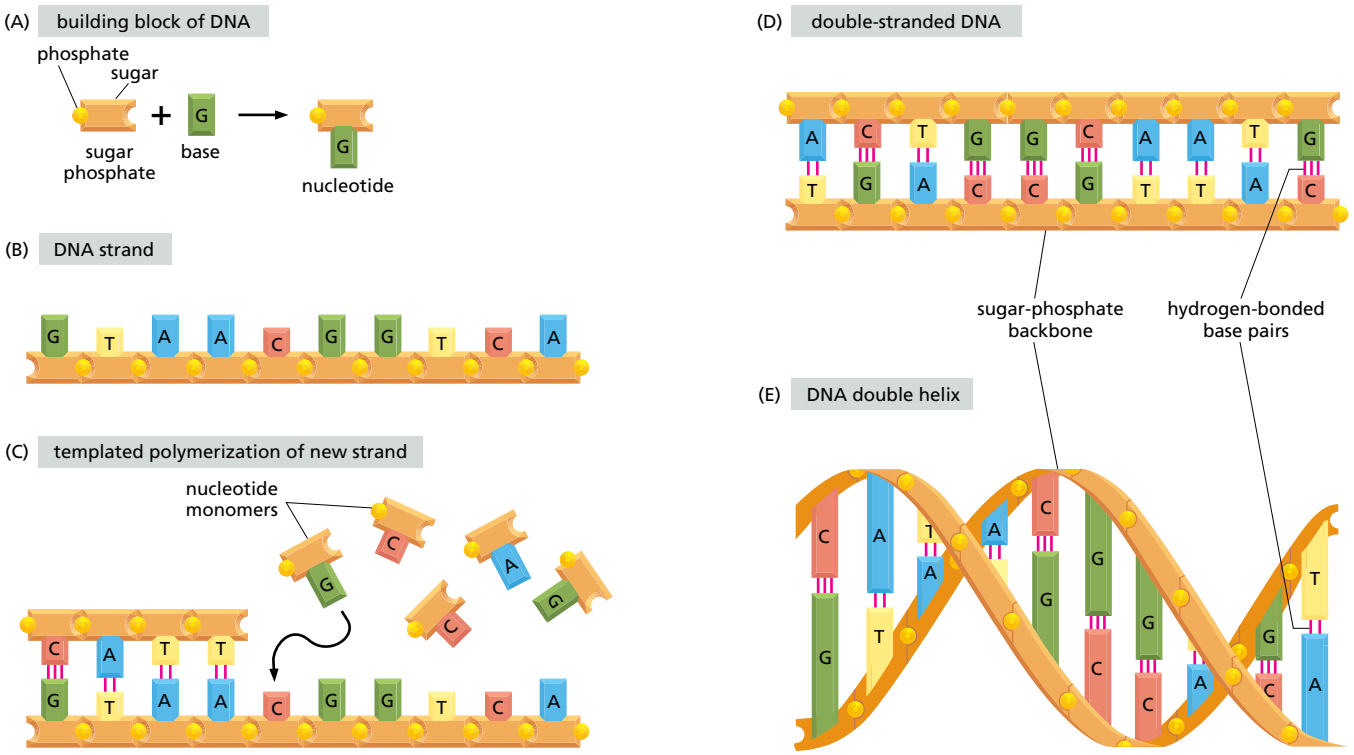


Figure 1–2 DNA and its building blocks. (A) DNA is made from simple subunits, called nucleotides, each consisting of a sugar-phosphate molecule with a nitrogen-containing sidegroup, or base, attached to it. The bases are of four types (adenine, guanine, cytosine, and thymine), corresponding to four distinct nucleotides, labeled A, G, C, and T. (B) A single strand of DNA consists of nucleotides joined together by sugar-phosphate linkages. Note that the individual sugar-phosphate units are asymmetric, giving the backbone of the strand a definite directionality, or polarity. This directionality guides the molecular processes by which the information in DNA is interpreted and copied in cells: the information is always "read" in a consistent order, just as written English text is read from left to right. (C) Through templated polymerization, the sequence of nucleotides in an existing DNA strand controls the sequence in which nucleotides are joined together in a new DNA strand; T in one strand pairs with A in the other, and G in one strand with C in the other. The new strand has a nucleotide sequence complementary to that of the old strand, and a backbone with opposite directionality: corresponding to the GTAA... of the original strand, it has ...TTAC. (D) A normal DNA molecule consists of two such complementary strands. The nucleotides within each strand are linked by strong (covalent) chemical bonds; the complementary nucleotides on opposite strands are held together more weakly, by hydrogen bonds. (E) The two strands twist around each other to form a double helix—a robust structure that can accommodate any sequence of nucleotides without altering its basic structure.

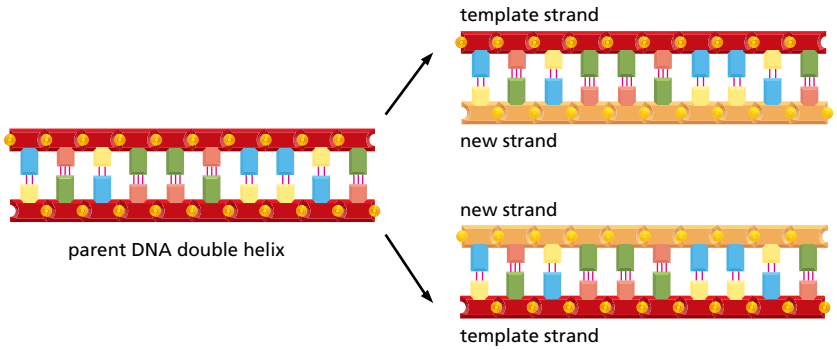


Figure 1–3 The copying of genetic information by DNA replication. In this process, the two strands of a DNA double helix are pulled apart, and each serves as a template for synthesis of a new complementary strand.

The bonds between the base pairs are weak compared with the sugar-phosphate links, and this allows the two DNA strands to be pulled apart without breakage of their backbones. Each strand then can serve as a template, in the way just described, for the synthesis of a fresh DNA strand complementary to itself—a fresh copy, that is, of the hereditary information (**Figure 1–3**). In different types of cells, this process of **DNA replication** occurs at different rates, with different controls to start it or stop it, and different auxiliary molecules to help it along. But the basics are universal: DNA is the information store, and *templated polymerization* is the way in which this information is copied throughout the living world.

All Cells Transcribe Portions of Their Hereditary Information into the Same Intermediary Form (RNA)

To carry out its information-bearing function, DNA must do more than copy itself. It must also *express* its information, by letting it guide the synthesis of other molecules in the cell. This also occurs by a mechanism that is the same in all living organisms, leading first and foremost to the production of two other key classes of polymers: RNAs and proteins. The process (discussed in detail in Chapters 6 and 7) begins with a templated polymerization called **transcription**, in which segments of the DNA sequence are used as templates for the synthesis of shorter molecules of the closely related polymer **ribonucleic acid**, or **RNA**. Later, in the more complex process of **translation**, many of these RNA molecules direct the synthesis of polymers of a radically different chemical class—the *proteins* (**Figure 1–4**).

In RNA, the backbone is formed of a slightly different sugar from that of DNA—ribose instead of deoxyribose—and one of the four bases is slightly different—uracil (U) in place of thymine (T); but the other three bases—A, C, and G—are the same, and all four bases pair with their complementary counterparts in DNA—the A, U, C, and G of RNA with the T, A, G, and C of DNA. During transcription, RNA monomers are lined up and selected for polymerization on a template strand of DNA, just as DNA monomers are selected during replication. The outcome is a polymer molecule whose sequence of nucleotides faithfully represents a part of the cell's genetic information, even though written in a slightly different alphabet, consisting of RNA monomers instead of DNA monomers.

The same segment of DNA can be used repeatedly to guide the synthesis of many identical RNA transcripts. Thus, whereas the cell's archive of genetic information in the form of DNA is fixed and sacrosanct, the RNA transcripts are mass-produced and disposable (**Figure 1–5**). As we shall see, these transcripts function as intermediates in the transfer of genetic information: they mainly serve as **messenger RNA** (**mRNA**) to guide the synthesis of proteins according to the genetic instructions stored in the DNA.

RNA molecules have distinctive structures that can also give them other specialized chemical capabilities. Being single-stranded, their backbone is flexible, so that the polymer chain can bend back on itself to allow one part of the

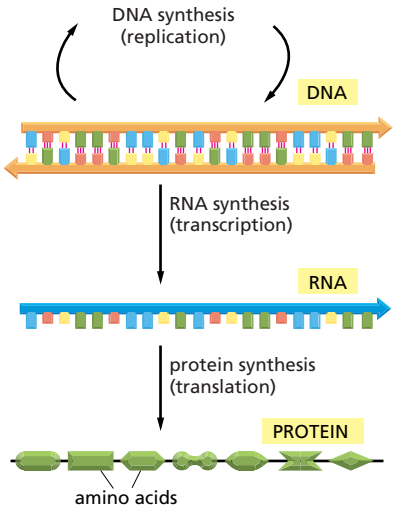


Figure 1–4 From DNA to protein. Genetic information is read out and put to use through a two-step process. First, in *transcription*, segments of the DNA sequence are used to guide the synthesis of molecules of RNA. Then, in *translation*, the RNA molecules are used to guide the synthesis of molecules of protein.

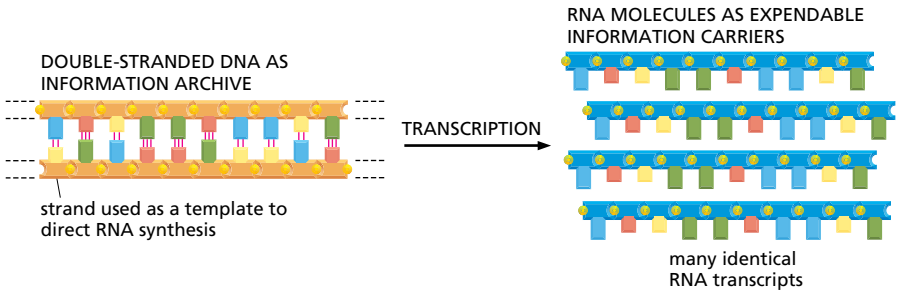


Figure 1–5 How genetic information is broadcast for use inside the cell. Each cell contains a fixed set of DNA molecules—its archive of genetic information. A given segment of this DNA guides the synthesis of many identical RNA transcripts, which serve as working copies of the information stored in the archive. Many different sets of RNA molecules can be made by transcribing selected parts of a long DNA sequence, allowing each cell to use its information store differently.

molecule to form weak bonds with another part of the same molecule. This occurs when segments of the sequence are locally complementary: a ...GGGG... segment, for example, will tend to associate with a ...CCCC... segment. These types of internal associations can cause an RNA chain to fold up into a specific shape that is dictated by its sequence (**Figure 1–6**). The shape of the RNA molecule, in turn, may enable it to recognize other molecules by binding to them selectively—and even, in certain cases, to catalyze chemical changes in the molecules that are bound. As we see in Chapter 6, a few chemical reactions catalyzed by RNA molecules are crucial for several of the most ancient and fundamental processes in living cells, and it has been suggested that more extensive catalysis by RNA played a central part in the early evolution of life.

All Cells Use Proteins as Catalysts

Protein molecules, like DNA and RNA molecules, are long unbranched polymer chains, formed by stringing together monomeric building blocks drawn from a standard repertoire that is the same for all living cells. Like DNA and RNA, they carry information in the form of a linear sequence of symbols, in the same way as a human message written in an alphabetic script. There are many different protein molecules in each cell, and—leaving out the water—they form most of the cell's mass.

The monomers of protein, the **amino acids**, are quite different from those of DNA and RNA, and there are 20 types, instead of 4. Each amino acid is built around the same core structure through which it can be linked in a standard way to any other amino acid in the set; attached to this core is a side group that gives each amino acid a distinctive chemical character. Each of the protein molecules, or **polypeptides**, created by joining amino acids in a particular sequence folds into a precise three-dimensional form with reactive sites on its surface (**Figure**

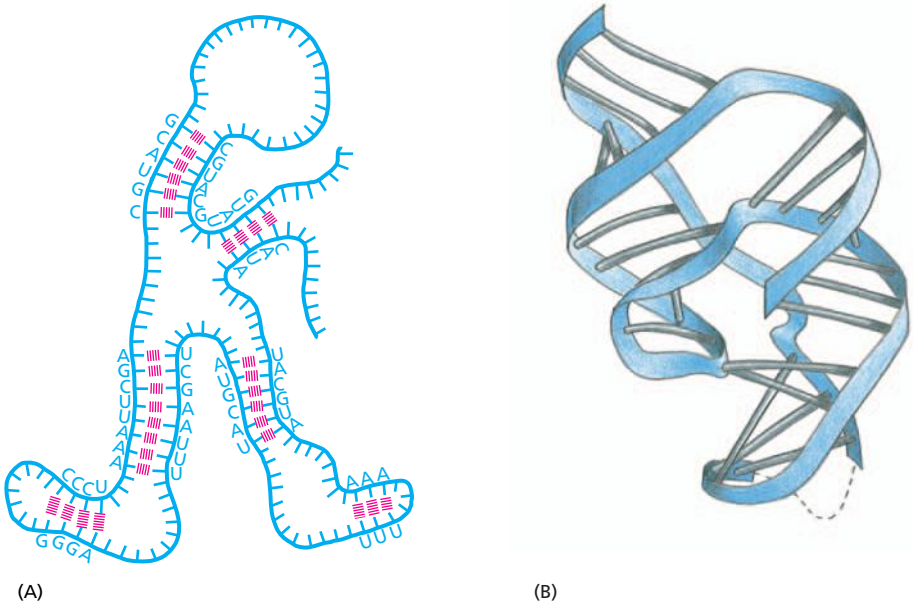


Figure 1–6 The conformation of an RNA molecule. (A) Nucleotide pairing between different regions of the same RNA polymer chain causes the molecule to adopt a distinctive shape. (B) The three-dimensional structure of an actual RNA molecule, from hepatitis delta virus, that catalyzes RNA strand cleavage. The *blue* ribbon represents the sugar-phosphate backbone; the bars represent base pairs. (B, based on A.R. Ferré D'Amaré, K. Zhou and J.A. Doudna, *Nature* 395:567–574, 1998. With permission from Macmillan Publishers Ltd.)

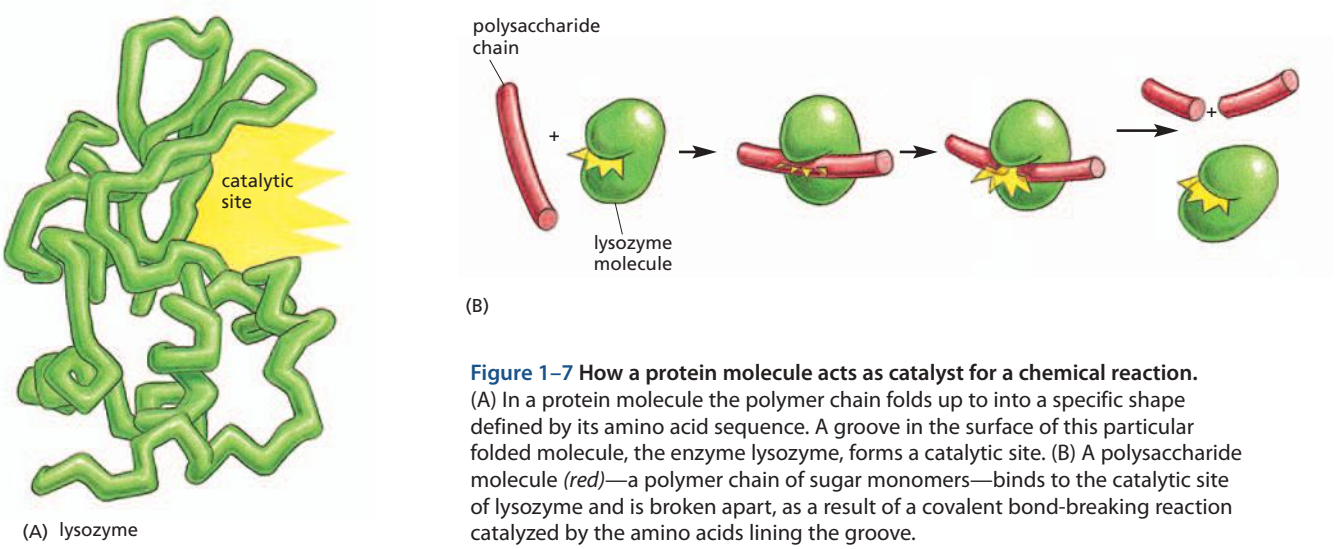


Figure 1–7 How a protein molecule acts as catalyst for a chemical reaction. (A) In a protein molecule the polymer chain folds up to into a specific shape defined by its amino acid sequence. A groove in the surface of this particular folded molecule, the enzyme lysozyme, forms a catalytic site. (B) A polysaccharide molecule *(red)*—a polymer chain of sugar monomers—binds to the catalytic site of lysozyme and is broken apart, as a result of a covalent bond-breaking reaction catalyzed by the amino acids lining the groove.

1–7A). These amino acid polymers thereby bind with high specificity to other molecules and act as **enzymes** to catalyze reactions that make or break covalent bonds. In this way they direct the vast majority of chemical processes in the cell (Figure 1–7B). Proteins have many other functions as well—maintaining structures, generating movements, sensing signals, and so on—each protein molecule performing a specific function according to its own genetically specified sequence of amino acids. Proteins, above all, are the molecules that put the cell's genetic information into action.

Thus, polynucleotides specify the amino acid sequences of proteins. Proteins, in turn, catalyze many chemical reactions, including those by which new DNA molecules are synthesized, and the genetic information in DNA is used to make both RNA and proteins. This feedback loop is the basis of the autocatalytic, self-reproducing behavior of living organisms (**Figure 1–8**).

All Cells Translate RNA into Protein in the Same Way

The translation of genetic information from the 4-letter alphabet of polynucleotides into the 20-letter alphabet of proteins is a complex process. The rules of this translation seem in some respects neat and rational, in other respects strangely arbitrary, given that they are (with minor exceptions) identical in all living things. These arbitrary features, it is thought, reflect frozen accidents in the early history of life—chance properties of the earliest organisms that were passed on by heredity and have become so deeply embedded in the constitution of all living cells that they cannot be changed without disastrous effects.

The information in the sequence of a messenger RNA molecule is read out in groups of three nucleotides at a time: each triplet of nucleotides, or *codon*, specifies (codes for) a single amino acid in a corresponding protein. Since there are 64 ($= 4 \times 4 \times 4$) possible codons, all of which occur in nature, but only 20 amino acids, there are necessarily many cases in which several codons correspond to the same amino acid. The code is read out by a special class of small RNA molecules, the **transfer RNAs** (**tRNAs**). Each type of tRNA becomes attached at one end to a specific amino acid, and displays at its other end a specific sequence of three nucleotides—an *anticodon*—that enables it to recognize, through base-pairing, a particular codon or subset of codons in mRNA (**Figure 1–9**).

For synthesis of protein, a succession of tRNA molecules charged with their appropriate amino acids have to be brought together with an mRNA molecule and matched up by base-pairing through their anticodons with each of its successive codons. The amino acids then have to be linked together to extend the growing protein chain, and the tRNAs, relieved of their burdens, have to be released. This whole complex of processes is carried out by a giant multimolecular machine, the ribosome, formed of two main chains of RNA, called **ribosomal RNAs**

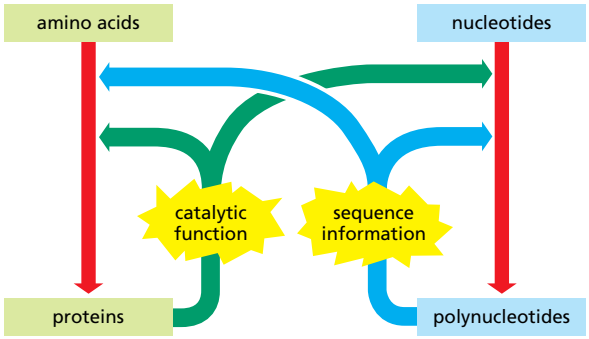


Figure 1–8 Life as an autocatalytic process. Polynucleotides (nucleotide polymers) and proteins (amino acid polymers) provide the sequence information and the catalytic functions that serve—through a complex set of chemical reactions—to bring about the synthesis of more polynucleotides and proteins of the same types.

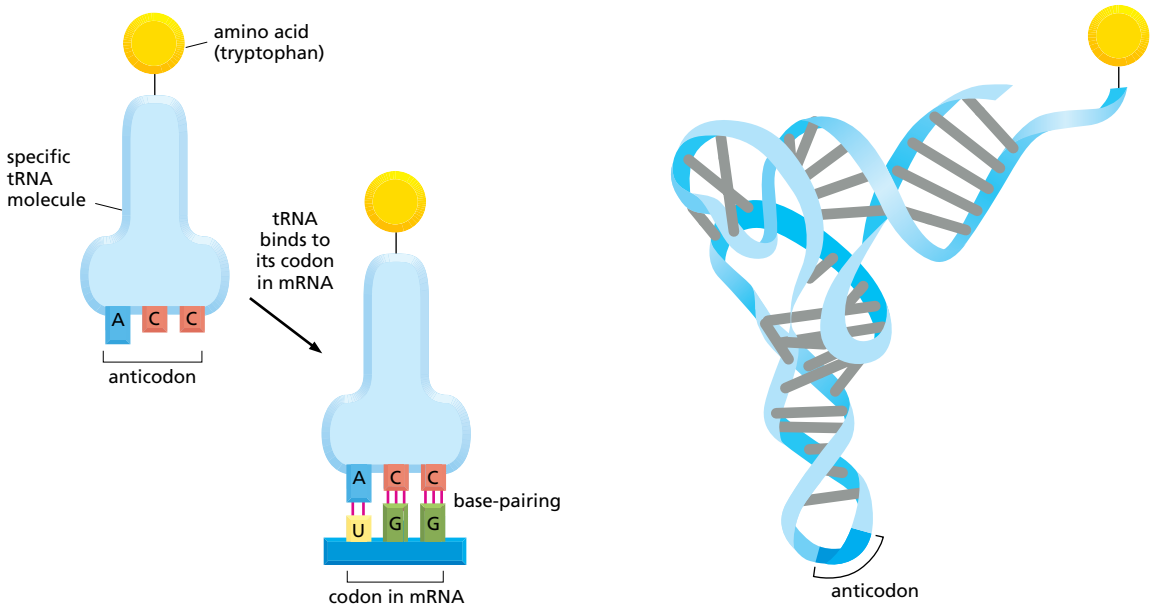
(**rRNAs**), and more than 50 different proteins. This evolutionarily ancient molecular juggernaut latches onto the end of an mRNA molecule and then trundles along it, capturing loaded tRNA molecules and stitching together the amino acids they carry to form a new protein chain (**Figure 1–10**).

The Fragment of Genetic Information Corresponding to One Protein Is One Gene

DNA molecules as a rule are very large, containing the specifications for thousands of proteins. Individual segments of the entire DNA sequence are transcribed into separate mRNA molecules, with each segment coding for a different protein. Each such DNA segment represents one **gene**. A complication is that RNA molecules transcribed from the same DNA segment can often be processed in more than one way, so as to give rise to a set of alternative versions of a protein, especially in more complex cells such as those of plants and animals. A gene therefore is defined, more generally, as the segment of DNA sequence corresponding to a single protein or set of alternative protein variants (or to a single catalytic or structural RNA molecule for those genes that produce RNA but not protein).

In all cells, the *expression* of individual genes is regulated: instead of manufacturing its full repertoire of possible proteins at full tilt all the time, the cell adjusts the rate of transcription and translation of different genes independently, according to need. Stretches of *regulatory DNA* are interspersed among the segments

Figure 1–9 Transfer RNA. (A) A tRNA molecule specific for the amino acid tryptophan. One end of the tRNA molecule has tryptophan attached to it, while the other end displays the triplet nucleotide sequence CCA (its anticodon), which recognizes the tryptophan codon in messenger RNA molecules. (B) The three-dimensional structure of the tryptophan tRNA molecule. Note that the codon and the anticodon in (A) are in antiparallel orientations, like the two strands in a DNA double helix (see Figure 1–2), so that the sequence of the anticodon in the tRNA is read from right to left, while that of the codon in the mRNA is read from left to right.



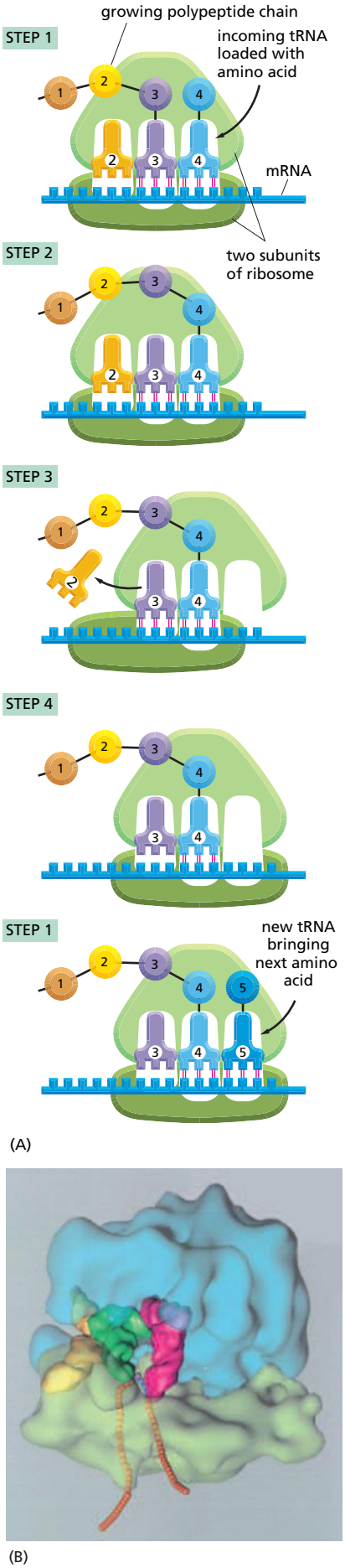


Figure 1–10 A ribosome at work. (A) The diagram shows how a ribosome moves along an mRNA molecule, capturing tRNA molecules that match the codons in the mRNA and using them to join amino acids into a protein chain. The mRNA specifies the sequence of amino acids. (B) The three-dimensional structure of a bacterial ribosome *(pale green* and *blue),* moving along an mRNA molecule *(orange* beads*),* with three tRNA molecules *(yellow*, *green*, and *pink)* at different stages in their process of capture and release. The ribosome is a giant assembly of more than 50 individual protein and RNA molecules. (B, courtesy of Joachim Frank, Yanhong Li and Rajendra Agarwal.)

that code for protein, and these noncoding regions bind to special protein molecules that control the local rate of transcription (**Figure 1–11**). Other noncoding DNA is also present, some of it serving, for example, as punctuation, defining where the information for an individual protein begins and ends. The quantity and organization of the regulatory and other noncoding DNA vary widely from one class of organisms to another, but the basic strategy is universal. In this way, the **genome** of the cell—that is, the total of its genetic information as embodied in its complete DNA sequence—dictates not only the nature of the cell's proteins, but also when and where they are to be made.

Life Requires Free Energy

A living cell is a dynamic chemical system, operating far from chemical equilibrium. For a cell to grow or to make a new cell in its own image, it must take in free energy from the environment, as well as raw materials, to drive the necessary synthetic reactions. This consumption of free energy is fundamental to life. When it stops, a cell decays towards chemical equilibrium and soon dies.

Genetic information is also fundamental to life. Is there any connection? The answer is yes: free energy is required for the propagation of information. For example, to specify one bit of information—that is, one yes/no choice between two equally probable alternatives—costs a defined amount of free energy that can be calculated. The quantitative relationship involves some deep reasoning and depends on a precise definition of the term "free energy," discussed in Chapter 2. The basic idea, however, is not difficult to understand intuitively.

Picture the molecules in a cell as a swarm of objects endowed with thermal energy, moving around violently at random, buffeted by collisions with one another. To specify genetic information—in the form of a DNA sequence, for example—molecules from this wild crowd must be captured, arranged in a specific order defined by some preexisting template, and linked together in a fixed relationship. The bonds that hold the molecules in their proper places on the template and join them together must be strong enough to resist the disordering effect of thermal motion. The process is driven forward by consumption of free energy, which is needed to ensure that the correct bonds are made, and made robustly. In the simplest case, the molecules can be compared with spring-loaded traps, ready to snap into a more stable, lower-energy attached state when they meet their proper partners; as they snap together into the bonded arrangement, their available stored energy—their free energy—like the energy of the spring in the trap, is released and dissipated as heat. In a cell, the chemical processes underlying information transfer are more complex, but the same basic principle applies: free energy has to be spent on the creation of order.

To replicate its genetic information faithfully, and indeed to make all its complex molecules according to the correct specifications, the cell therefore requires free energy, which has to be imported somehow from the surroundings.

All Cells Function as Biochemical Factories Dealing with the Same Basic Molecular Building Blocks

Because all cells make DNA, RNA, and protein, and these macromolecules are composed of the same set of subunits in every case, all cells have to contain and

manipulate a similar collection of small molecules, including simple sugars, nucleotides, and amino acids, as well as other substances that are universally required for their synthesis. All cells, for example, require the phosphorylated nucleotide *ATP (adenosine triphosphate)* as a building block for the synthesis of DNA and RNA; and all cells also make and consume this molecule as a carrier of free energy and phosphate groups to drive many other chemical reactions.

Although all cells function as biochemical factories of a broadly similar type, many of the details of their small-molecule transactions differ, and it is not as easy as it is for the informational macromolecules to point out the features that are strictly universal. Some organisms, such as plants, require only the simplest of nutrients and harness the energy of sunlight to make from these almost all their own small organic molecules; other organisms, such as animals, feed on living things and obtain many of their organic molecules ready-made. We return to this point below.

All Cells Are Enclosed in a Plasma Membrane Across Which Nutrients and Waste Materials Must Pass

There is, however, at least one other feature of cells that is universal: each one is enclosed by a membrane—the **plasma membrane**. This container acts as a selective barrier that enables the cell to concentrate nutrients gathered from its environment and retain the products it synthesizes for its own use, while excreting its waste products. Without a plasma membrane, the cell could not maintain its integrity as a coordinated chemical system.

The molecules forming this membrane have the simple physico-chemical property of being *amphiphilic*—that is, consisting of one part that is hydrophobic (water-insoluble) and another part that is hydrophilic (water-soluble). Such molecules placed in water aggregate spontaneously, arranging their hydrophobic portions to be as much in contact with one another as possible to hide them from the water, while keeping their hydrophilic portions exposed. Amphiphilic molecules of appropriate shape, such as the phospholipid molecules that comprise most of the plasma membrane, spontaneously aggregate in water to form a *bilayer* that creates small closed vesicles (**Figure 1–12**). The phenomenon can be demonstrated in a test tube by simply mixing phospholipids and water together; under appropriate conditions, small vesicles form whose aqueous contents are isolated from the external medium.

Although the chemical details vary, the hydrophobic tails of the predominant membrane molecules in all cells are hydrocarbon polymers ($–CH_2–CH_2–CH_2–$), and their spontaneous assembly into a bilayered vesicle is but one of many examples of an important general principle: cells produce molecules whose chemical properties cause them to *self-assemble* into the structures that a cell needs.

The cell boundary cannot be totally impermeable. If a cell is to grow and reproduce, it must be able to import raw materials and export waste across its plasma membrane. All cells therefore have specialized proteins embedded in their membrane that transport specific molecules from one side to the other (**Figure 1–13**). Some of these *membrane transport proteins*, like some of the proteins that catalyze the fundamental small-molecule reactions inside the cell,

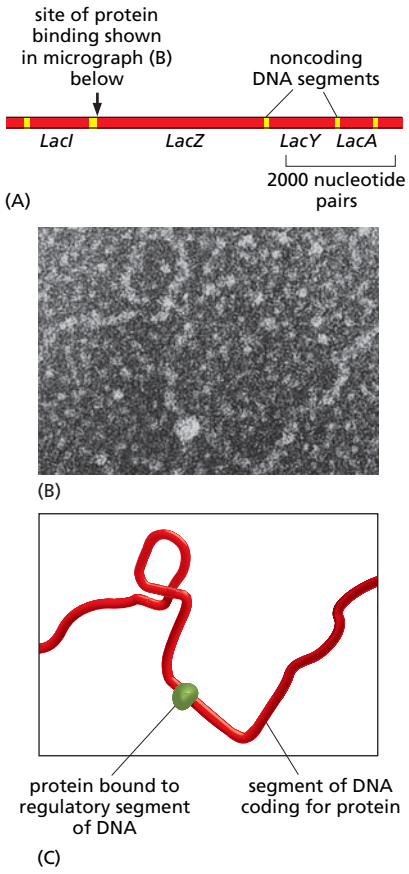


Figure 1–11 Gene regulation by protein binding to regulatory DNA. (A) A diagram of a small portion of the genome of the bacterium *Escherichia coli,* containing genes (called *LacI, LacZ, LacY,* and *LacA)* coding for four different proteins. The protein-coding DNA segments *(red)* have regulatory and other noncoding DNA segments *(yellow)* between them. (B) An electron micrograph of DNA from this region, with a protein molecule (encoded by the *LacI* gene) bound to the regulatory segment; this protein controls the rate of transcription of the *LacZ*, *LacY*, and *LacA* genes. (C) A drawing of the structures shown in (B). (B, courtesy of Jack Griffith.)

have been so well preserved over the course of evolution that we can recognize the family resemblances between them in comparisons of even the most distantly related groups of living organisms.

The transport proteins in the membrane largely determine which molecules enter the cell, and the catalytic proteins inside the cell determine the reactions that those molecules undergo. Thus, by specifying the proteins that the cell is to manufacture, the genetic information recorded in the DNA sequence dictates the entire chemistry of the cell; and not only its chemistry, but also its form and its behavior, for these too are chiefly constructed and controlled by the cell's proteins.

A Living Cell Can Exist with Fewer Than 500 Genes

The basic principles of biological information transfer are simple enough, but how complex are real living cells? In particular, what are the minimum requirements? We can get a rough indication by considering a species that has one of the smallest known genomes—the bacterium *Mycoplasma genitalium* (**Figure 1–14**). This organism lives as a parasite in mammals, and its environment provides it with many of its small molecules ready-made. Nevertheless, it still has to make all the large molecules—DNA, RNAs, and proteins—required for the basic processes of heredity. It has only about 480 genes in its genome of 580,070 nucleotide pairs, representing 145,018 bytes of information—about as much as it takes to record the text of one chapter of this book. Cell biology may be complicated, but it is not impossibly so.

The minimum number of genes for a viable cell in today's environments is probably not less than 200–300, although there are only about 60 genes in the core set shared by all living species without any known exception.

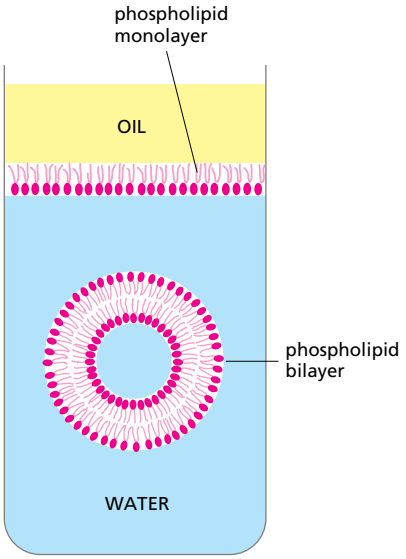


Figure 1–12 Formation of a membrane by amphiphilic phospholipid molecules. These have a hydrophilic (water-loving, phosphate) head group and a hydrophobic (water-avoiding, hydrocarbon) tail. At an interface between oil and water, they arrange themselves as a single sheet with their head groups facing the water and their tail groups facing the oil. When immersed in water, they aggregate to form bilayers enclosing aqueous compartments.

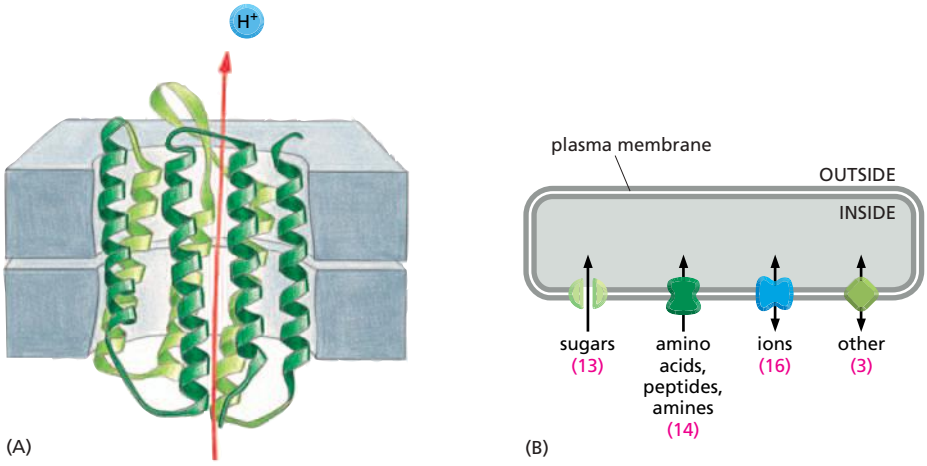


Figure 1–13 Membrane transport proteins. (A) Structure of a molecule of bacteriorhodopsin, from the archaeon (archaebacterium) *Halobacterium halobium*. This transport protein uses the energy of absorbed light to pump protons (H^+ ions) out of the cell. The polypeptide chain threads to and fro across the membrane; in several regions it is twisted into a helical conformation, and the helical segments are arranged to form the walls of a channel through which ions are transported. (B) Diagram of the set of transport proteins found in the membrane of the bacterium *Thermotoga maritima*. The numbers in parentheses refer to the number of different membrane transport proteins of each type. Most of the proteins within each class are evolutionarily related to one another and to their counterparts in other species.

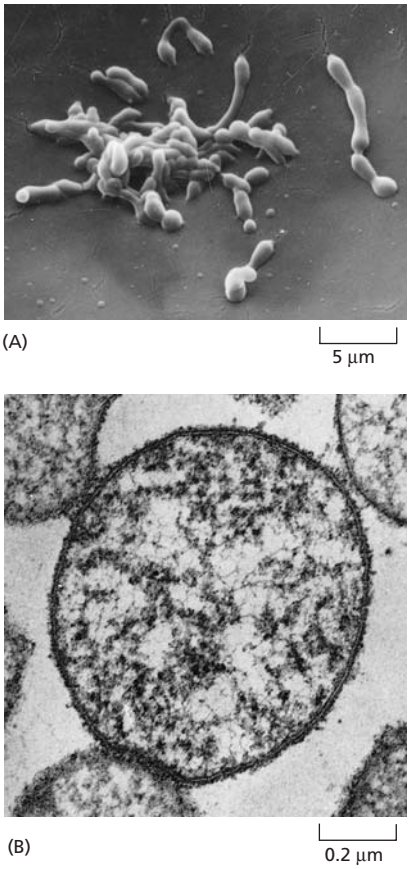


Figure 1–14 Mycoplasma genitalium. (A) Scanning electron micrograph showing the irregular shape of this small bacterium, reflecting the lack of any rigid wall. (B) Cross section (transmission electron micrograph) of a *Mycoplasma* cell. Of the 477 genes of *Mycoplasma genitalium*, 37 code for transfer, ribosomal, and other nonmessenger RNAs. Functions are known, or can be guessed, for 297 of the genes coding for protein: of these, 153 are involved in replication, transcription, translation, and related processes involving DNA, RNA, and protein; 29 in the membrane and surface structures of the cell; 33 in the transport of nutrients and other molecules across the membrane; 71 in energy conversion and the synthesis and degradation of small molecules; and 11 in the regulation of cell division and other processes. (A, from S. Razin et al., *Infect. Immun.* 30:538–546, 1980. With permission from the American Society for Microbiology; B, courtesy of Roger Cole, in Medical Microbiology, 4th ed. [S. Baron ed.]. Galveston: University of Texas Medical Branch, 1996.)

Summary

Living organisms reproduce themselves by transmitting genetic information to their progeny. The individual cell is the minimal self-reproducing unit, and is the vehicle for transmission of the genetic information in all living species. Every cell on our planet stores its genetic information in the same chemical form—as double-stranded DNA. The cell replicates its information by separating the paired DNA strands and using each as a template for polymerization to make a new DNA strand with a complementary sequence of nucleotides. The same strategy of templated polymerization is used to transcribe portions of the information from DNA into molecules of the closely related polymer, RNA. These in turn guide the synthesis of protein molecules by the more complex machinery of translation, involving a large multimolecular machine, the ribosome, which is itself composed of RNA and protein. Proteins are the principal catalysts for almost all the chemical reactions in the cell; their other functions include the selective import and export of small molecules across the plasma membrane that forms the cell's boundary. The specific function of each protein depends on its amino acid sequence, which is specified by the nucleotide sequence of a corresponding segment of the DNA—the gene that codes for that protein. In this way, the genome of the cell determines its chemistry; and the chemistry of every living cell is fundamentally similar, because it must provide for the synthesis of DNA, RNA, and protein. The simplest known cells have just under 500 genes.

THE DIVERSITY OF GENOMES AND THE TREE OF LIFE

The success of living organisms based on DNA, RNA, and protein, out of the infinitude of other chemical forms that we might conceive of, has been spectacular. They have populated the oceans, covered the land, infiltrated the Earth's crust, and molded the surface of our planet. Our oxygen-rich atmosphere, the deposits of coal and oil, the layers of iron ores, the cliffs of chalk and limestone and marble—all these are products, directly or indirectly, of past biological activity on Earth.

Living things are not confined to the familiar temperate realm of land, water, and sunlight inhabited by plants and plant-eating animals. They can be found in the darkest depths of the ocean, in hot volcanic mud, in pools beneath the frozen surface of the Antarctic, and buried kilometers deep in the Earth's crust. The creatures that live in these extreme environments are generally unfamiliar, not only because they are inaccessible, but also because they are mostly microscopic. In more homely habitats, too, most organisms are too small for us to see without special equipment: they tend to go unnoticed, unless they cause a disease or rot the timbers of our houses. Yet microorganisms make up most of the

total mass of living matter on our planet. Only recently, through new methods of molecular analysis and specifically through the analysis of DNA sequences, have we begun to get a picture of life on Earth that is not grossly distorted by our biased perspective as large animals living on dry land.

In this section we consider the diversity of organisms and the relationships among them. Because the genetic information for every organism is written in the universal language of DNA sequences, and the DNA sequence of any given organism can be obtained by standard biochemical techniques, it is now possible to characterize, catalogue, and compare any set of living organisms with reference to these sequences. From such comparisons we can estimate the place of each organism in the family tree of living species—the 'tree of life'. But before describing what this approach reveals, we need first to consider the routes by which cells in different environments obtain the matter and energy they require to survive and proliferate, and the ways in which some classes of organisms depend on others for their basic chemical needs.

Cells Can Be Powered by a Variety of Free Energy Sources

Living organisms obtain their free energy in different ways. Some, such as animals, fungi, and the bacteria that live in the human gut, get it by feeding on other living things or the organic chemicals they produce; such organisms are called *organotrophic* (from the Greek word *trophe*, meaning "food"). Others derive their energy directly from the nonliving world. These fall into two classes: those that harvest the energy of sunlight, and those that capture their energy from energy-rich systems of inorganic chemicals in the environment (chemical systems that are far from chemical equilibrium). Organisms of the former class are called *phototrophic* (feeding on sunlight); those of the latter are called *lithotrophic* (feeding on rock). Organotrophic organisms could not exist without these primary energy converters, which are the most plentiful form of life.

Phototrophic organisms include many types of bacteria, as well as algae and plants, on which we—and virtually all the living things that we ordinarily see around us—depend. Phototrophic organisms have changed the whole chemistry of our environment: the oxygen in the Earth's atmosphere is a by-product of their biosynthetic activities.

Lithotrophic organisms are not such an obvious feature of our world, because they are microscopic and mostly live in habitats that humans do not frequent—deep in the ocean, buried in the Earth's crust, or in various other inhospitable environments. But they are a major part of the living world, and are especially important in any consideration of the history of life on Earth.

Some lithotrophs get energy from *aerobic* reactions, which use molecular oxygen from the environment; since atmospheric O_2 is ultimately the product of living organisms, these aerobic lithotrophs are, in a sense, feeding on the products of past life. There are, however, other lithotrophs that live anaerobically, in places where little or no molecular oxygen is present, in circumstances similar to those that must have existed in the early days of life on Earth, before oxygen had accumulated.

The most dramatic of these sites are the hot *hydrothermal vents* found deep down on the floor of the Pacific and Atlantic Oceans, in regions where the ocean floor is spreading as new portions of the Earth's crust form by a gradual upwelling of material from the Earth's interior (**Figure 1–15**). Downward-percolating seawater is heated and driven back upward as a submarine geyser, carrying with it a current of chemicals from the hot rocks below. A typical cocktail might include H_2S, H_2, CO, Mn^{2+}, Fe^{2+}, Ni^{2+}, CH_2, NH_4^+, and phosphorus-containing compounds. A dense population of microbes lives in the neighborhood of the vent, thriving on this austere diet and harvesting free energy from reactions between the available chemicals. Other organisms—clams, mussels, and giant marine worms—in turn live off the microbes at the vent, forming an entire ecosystem analogous to the system of plants and animals that we belong to, but powered by geochemical energy instead of light (**Figure 1–16**).

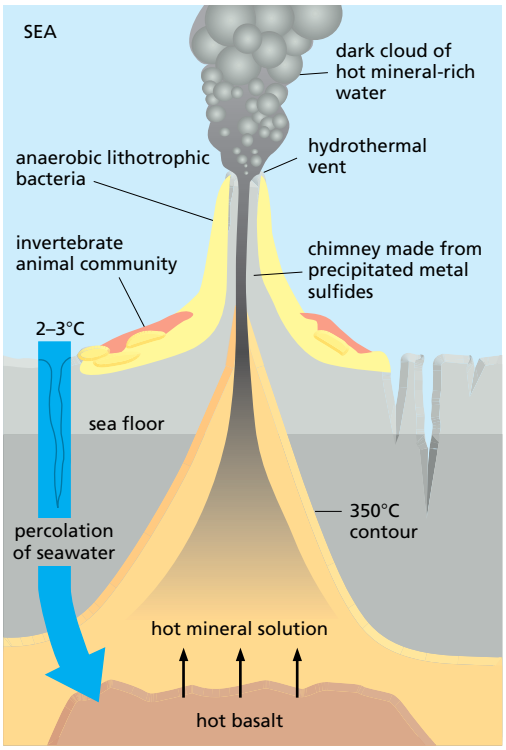


Figure 1–15 The geology of a hot hydrothermal vent in the ocean floor. Water percolates down toward the hot molten rock upwelling from the Earth's interior and is heated and driven back upward, carrying minerals leached from the hot rock. A temperature gradient is set up, from more than 350°C near the core of the vent, down to 2–3°C in the surrounding ocean. Minerals precipitate from the water as it cools, forming a chimney. Different classes of organisms, thriving at different temperatures, live in different neighborhoods of the chimney. A typical chimney might be a few meters tall, with a flow rate of 1–2 m/sec.

Some Cells Fix Nitrogen and Carbon Dioxide for Others

To make a living cell requires matter, as well as free energy. DNA, RNA, and protein are composed of just six elements: hydrogen, carbon, nitrogen, oxygen, sulfur, and phosphorus. These are all plentiful in the nonliving environment, in the Earth's rocks, water, and atmosphere, but not in chemical forms that allow easy incorporation into biological molecules. Atmospheric N_2 and CO_2, in particular, are extremely unreactive, and a large amount of free energy is required to drive the reactions that use these inorganic molecules to make the organic compounds needed for further biosynthesis—that is, to *fix* nitrogen and carbon dioxide, so as to make N and C available to living organisms. Many types of living cells lack the biochemical machinery to achieve this fixation, and rely on other classes of cells to do the job for them. We animals depend on plants for our supplies of

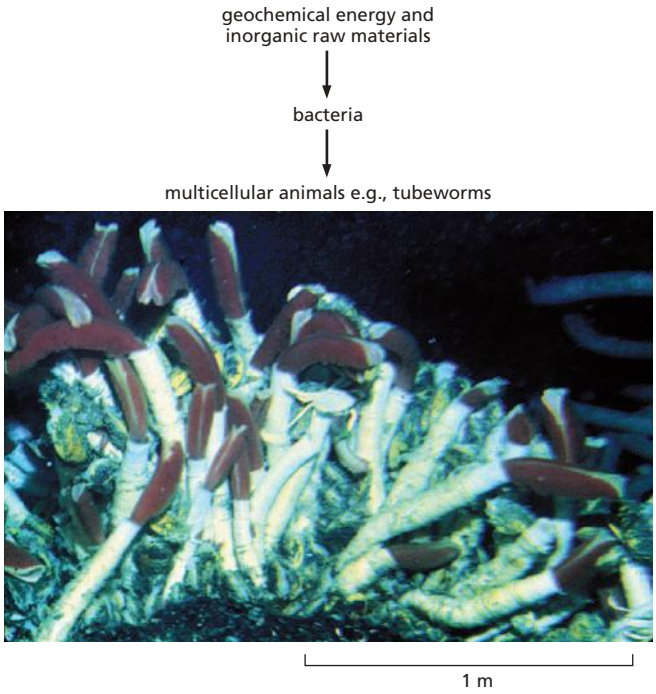


Figure 1–16 Living organisms at a hot hydrothermal vent. Close to the vent, at temperatures up to about 120°C, various lithotrophic species of bacteria and archaea (archaebacteria) live, directly fuelled by geochemical energy. A little further away, where the temperature is lower, various invertebrate animals live by feeding on these microorganisms. Most remarkable are the giant (2-meter) tube worms, which, rather than feed on the lithotrophic cells, live in symbiosis with them: specialized organs in the worms harbor huge numbers of symbiotic sulfur-oxidizing bacteria. These bacteria harness geochemical energy and supply nourishment to their hosts, which have no mouth, gut, or anus. The dependence of the tube worms on the bacteria for the harnessing of geothermal energy is analogous to the dependence of plants on chloroplasts for the harnessing of solar energy, discussed later in this chapter. The tube worms, however, are thought to have evolved from more conventional animals, and to have become secondarily adapted to life at hydrothermal vents. (Courtesy of Dudley Foster, Woods Hole Oceanographic Institution.)

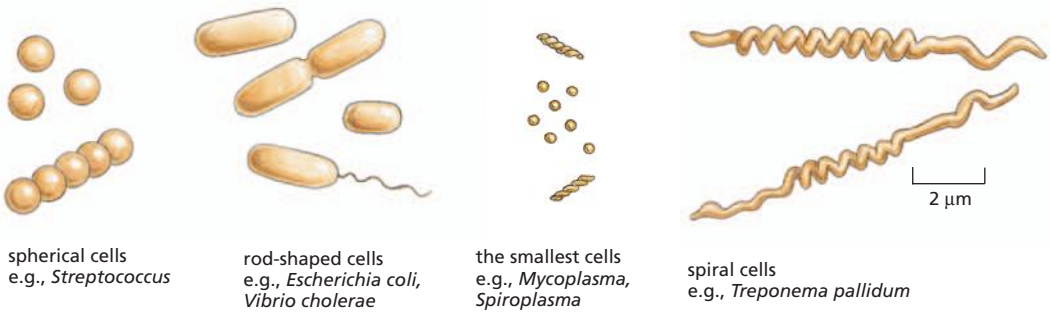


Figure 1–17 Shapes and sizes of some bacteria. Although most are small, as shown, measuring a few micrometers in linear dimension, there are also some giant species. An extreme example (not shown) is the cigar-shaped bacterium *Epulopiscium fishelsoni*, which lives in the gut of a surgeonfish and can be up to 600 μm long.

organic carbon and nitrogen compounds. Plants in turn, although they can fix carbon dioxide from the atmosphere, lack the ability to fix atmospheric nitrogen, and they depend in part on nitrogen-fixing bacteria to supply their need for nitrogen compounds. Plants of the pea family, for example, harbor symbiotic nitrogen-fixing bacteria in nodules in their roots.

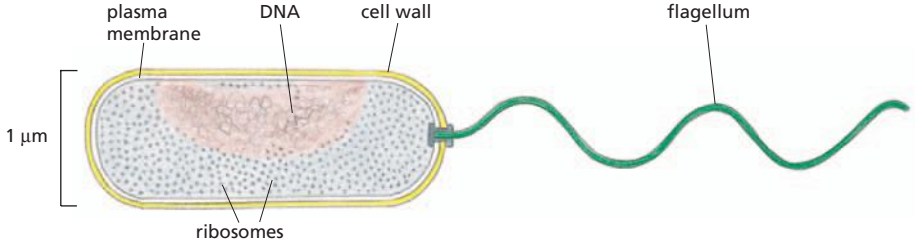
Living cells therefore differ widely in some of the most basic aspects of their biochemistry. Not surprisingly, cells with complementary needs and capabilities have developed close associations. Some of these associations, as we see below, have evolved to the point where the partners have lost their separate identities altogether: they have joined forces to form a single composite cell.

The Greatest Biochemical Diversity Exists Among Procaryotic Cells

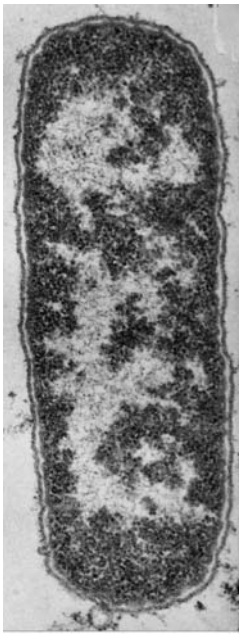
From simple microscopy, it has long been clear that living organisms can be classified on the basis of cell structure into two groups: the **eucaryotes** and the **procaryotes**. Eucaryotes keep their DNA in a distinct membrane-enclosed intracellular compartment called the nucleus. (The name is from the Greek, meaning "truly nucleated," from the words *eu*, "well" or "truly," and *karyon*, "kernel" or "nucleus".) Procaryotes have no distinct nuclear compartment to house their DNA. Plants, fungi, and animals are eucaryotes; bacteria are procaryotes, as are archaea—a separate class of procaryotic cells, discussed below.

Most procaryotic cells are small and simple in outward appearance (**Figure 1–17**), and they live mostly as independent individuals or in loosely organized communities, rather than as multicellular organisms. They are typically spherical or rod-shaped and measure a few micrometers in linear dimension. They often have a tough protective coat, called a *cell wall,* beneath which a plasma membrane encloses a single cytoplasmic compartment containing DNA, RNA, proteins, and the many small molecules needed for life. In the electron microscope, this cell interior appears as a matrix of varying texture without any discernible organized internal structure (**Figure 1–18**).

Figure 1–18 The structure of a bacterium. (A) The bacterium *Vibrio cholerae,* showing its simple internal organization. Like many other species, *Vibrio* has a helical appendage at one end—a flagellum—that rotates as a propeller to drive the cell forward. (B) An electron micrograph of a longitudinal section through the widely studied bacterium *Escherichia coli (E. coli).* This is related to *Vibrio* but has many flagella (not visible in this section) distributed over its surface. The cell's DNA is concentrated in the lightly stained region. (B, courtesy of E. Kellenberger.)



(A)



(B)

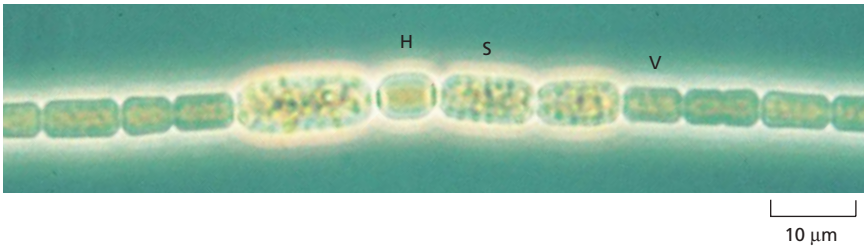


Figure 1–19 The phototrophic bacterium *Anabaena cylindrica* viewed in the light microscope. The cells of this species form long, multicellular filaments. Most of the cells (labeled *V)* perform photosynthesis, while others become specialized for nitrogen fixation (labeled *H),* or develop into resistant spores (labeled *S).* (Courtesy of Dave G. Adams.)

Procaryotic cells live in an enormous variety of ecological niches, and they are astonishingly varied in their biochemical capabilities—far more so than eucaryotic cells. Organotrophic species can utilize virtually any type of organic molecule as food, from sugars and amino acids to hydrocarbons and methane gas. Phototrophic species (**Figure 1–19**) harvest light energy in a variety of ways, some of them generating oxygen as a byproduct, others not. Lithotrophic species can feed on a plain diet of inorganic nutrients, getting their carbon from CO_2, and relying on H_2S to fuel their energy needs (**Figure 1–20**)—or on H_2, or Fe^{2+}, or elemental sulfur, or any of a host of other chemicals that occur in the environment.

Many parts of this world of microscopic organisms are virtually unexplored. Traditional methods of bacteriology have given us an acquaintance with those species that can be isolated and cultured in the laboratory. But DNA sequence analysis of the populations of bacteria in samples from natural habitats—such as soil or ocean water, or even the human mouth—has opened our eyes to the fact that most species cannot be cultured by standard laboratory techniques. According to one estimate, at least 99% of procaryotic species remain to be characterized.

The Tree of Life Has Three Primary Branches: Bacteria, Archaea, and Eucaryotes

The classification of living things has traditionally depended on comparisons of their outward appearances: we can see that a fish has eyes, jaws, backbone, brain, and so on, just as we do, and that a worm does not; that a rosebush is cousin to an apple tree, but less similar to a grass. As Darwin showed, we can readily interpret such close family resemblances in terms of evolution from common ancestors, and we can find the remains of many of these ancestors preserved in the fossil record. In this way, it has been possible to begin to draw a family tree of living organisms, showing the various lines of descent, as well as branch points in the history, where the ancestors of one group of species became different from those of another.

When the disparities between organisms become very great, however, these methods begin to fail. How do we decide whether a fungus is closer kin to a plant or to an animal? When it comes to procaryotes, the task becomes harder still: one microscopic rod or sphere looks much like another. Microbiologists have therefore sought to classify procaryotes in terms of their biochemistry and nutritional requirements. But this approach also has its pitfalls. Amid the bewildering variety of biochemical behaviors, it is difficult to know which differences truly reflect differences of evolutionary history.

Genome analysis has given us a simpler, more direct, and more powerful way to determine evolutionary relationships. The complete DNA sequence of an organism defines its nature with almost perfect precision and in exhaustive detail. Moreover, this specification is in a digital form—a string of letters—that can be entered straightforwardly into a computer and compared with the corresponding information for any other living thing. Because DNA is subject to random changes that accumulate over long periods of time (as we shall see shortly), the number of differences between the DNA sequences of two organisms can provide a direct, objective, quantitative indication of the evolutionary distance between them.

This approach has shown that the organisms that were traditionally classed together as "bacteria" can be as widely divergent in their evolutionary origins as



Figure 1–20 A lithotrophic bacterium. *Beggiatoa*, which lives in sulfurous environments, gets its energy by oxidizing H_2S and can fix carbon even in the dark. Note the yellow deposits of sulfur inside the cells. (Courtesy of Ralph W. Wolfe.)

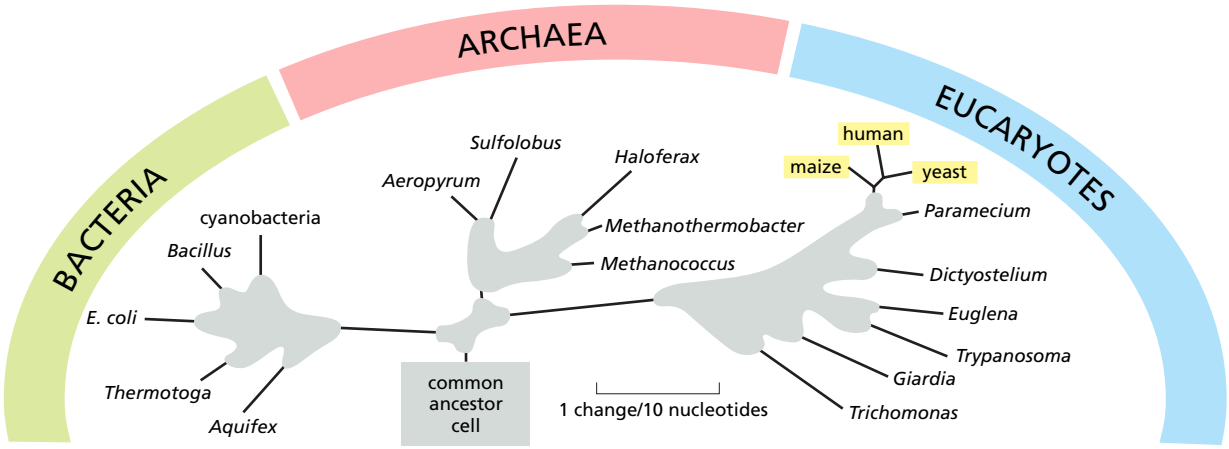


Figure 1–21 The three major divisions (domains) of the living world. Note that traditionally the word *bacteria* has been used to refer to procaryotes in general, but more recently has been redefined to refer to eubacteria specifically. The tree shown here is based on comparisons of the nucleotide sequence of a ribosomal RNA subunit in the different species, and the distances in the diagram represent estimates of the numbers of evolutionary changes that have occurred in this molecule in each lineage (see Figure 1–22). The parts of the tree shrouded in *gray cloud* represent uncertainties about details of the true pattern of species divergence in the course of evolution: comparisons of nucleotide or amino acid sequences of molecules other than rRNA, as well as other arguments, lead to somewhat different trees. There is general agreement, however, as to the early divergence of the three most basic domains—the bacteria, the archaea, and the eucaryotes.

is any procaryote from any eucaryote. It now appears that the procaryotes comprise two distinct groups that diverged early in the history of life on Earth, either before the ancestors of the eucaryotes diverged as a separate group or at about the same time. The two groups of procaryotes are called the **bacteria** (or eubacteria) and the **archaea** (or archaebacteria). The living world therefore has three major divisions or *domains*: bacteria, archaea, and eucaryotes (**Figure 1–21**).

Archaea are often found inhabiting environments that we humans avoid, such as bogs, sewage treatment plants, ocean depths, salt brines, and hot acid springs, although they are also widespread in less extreme and more homely environments, from soils and lakes to the stomachs of cattle. In outward appearance they are not easily distinguished from bacteria. At a molecular level, archaea seem to resemble eucaryotes more closely in their machinery for handling genetic information (replication, transcription, and translation), but bacteria more closely in their apparatus for metabolism and energy conversion. We discuss below how this might be explained.

Some Genes Evolve Rapidly; Others Are Highly Conserved

Both in the storage and in the copying of genetic information, random accidents and errors occur, altering the nucleotide sequence—that is, creating **mutations**. Therefore, when a cell divides, its two daughters are often not quite identical to one another or to their parent. On rare occasions, the error may represent a change for the better; more probably, it will cause no significant difference in the cell's prospects; and in many cases, the error will cause serious damage—for example, by disrupting the coding sequence for a key protein. Changes due to mistakes of the first type will tend to be perpetuated, because the altered cell has an increased likelihood of reproducing itself. Changes due to mistakes of the second type—*selectively neutral* changes—may be perpetuated or not: in the competition for limited resources, it is a matter of chance whether the altered cell or its cousins will succeed. But changes that cause serious damage lead nowhere: the cell that suffers them dies, leaving no progeny. Through endless repetition of this cycle of error and trial—of *mutation* and *natural selection*—

organisms evolve: their genetic specifications change, giving them new ways to exploit the environment more effectively, to survive in competition with others, and to reproduce successfully.

Clearly, some parts of the genome change more easily than others in the course of evolution. A segment of DNA that does not code for protein and has no significant regulatory role is free to change at a rate limited only by the frequency of random errors. In contrast, a gene that codes for a highly optimized essential protein or RNA molecule cannot alter so easily: when mistakes occur, the faulty cells are almost always eliminated. Genes of this latter sort are therefore *highly conserved*. Through 3.5 billion years or more of evolutionary history, many features of the genome have changed beyond all recognition; but the most highly conserved genes remain perfectly recognizable in all living species.

These latter genes are the ones we must examine if we wish to trace family relationships between the most distantly related organisms in the tree of life. The studies that led to the classification of the living world into the three domains of bacteria, archaea, and eucaryotes were based chiefly on analysis of one of the two main RNA components of the ribosome—the so-called small-subunit ribosomal RNA. Because translation is fundamental to all living cells, this component of the ribosome has been well conserved since early in the history of life on Earth (**Figure 1–22**).

Most Bacteria and Archaea Have 1000–6000 Genes

Natural selection has generally favored those procaryotic cells that can reproduce the fastest by taking up raw materials from their environment and replicating themselves most efficiently, at the maximal rate permitted by the available food supplies. Small size implies a large ratio of surface area to volume, thereby helping to maximize the uptake of nutrients across the plasma membrane and boosting a cell's reproductive rate.

Presumably for these reasons, most procaryotic cells carry very little superfluous baggage; their genomes are small, with genes packed closely together and minimal quantities of regulatory DNA between them. The small genome size makes it relatively easy to determine the complete DNA sequence. We now have this information for many species of bacteria and archaea, and a few species of eucaryotes. As shown in **Table 1–1**, most bacterial and archaeal genomes contain between 10^6 and 10^7 nucleotide pairs, encoding 1000–6000 genes.

A complete DNA sequence reveals both the genes an organism possesses and the genes it lacks. When we compare the three domains of the living world, we can begin to see which genes are common to all of them and must therefore have been present in the cell that was ancestral to all present-day living things, and which genes are peculiar to a single branch in the tree of life. To explain the findings, however, we need to consider a little more closely how new genes arise and genomes evolve.

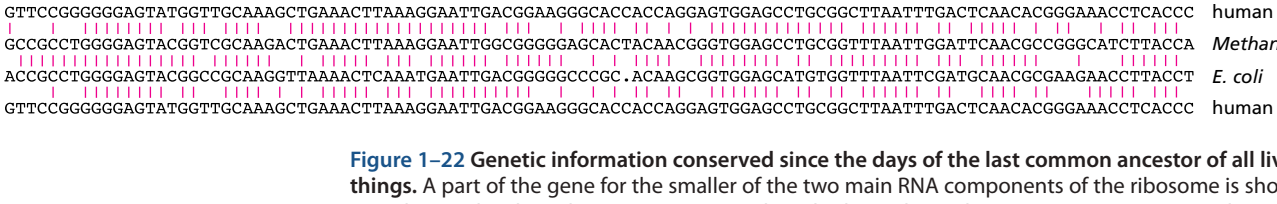


Figure 1–22 Genetic information conserved since the days of the last common ancestor of all living things. A part of the gene for the smaller of the two main RNA components of the ribosome is shown. (The complete molecule is about 1500–1900 nucleotides long, depending on species.) Corresponding segments of nucleotide sequence from an archaean *(Methanococcus jannaschii),* a bacterium *(Escherichia coli)* and a eucaryote *(Homo sapiens)* are aligned. Sites where the nucleotides are identical between species are indicated by a vertical line; the human sequence is repeated at the bottom of the alignment so that all three two-way comparisons can be seen. A dot halfway along the *E. coli* sequence denotes a site where a nucleotide has been either deleted from the bacterial lineage in the course of evolution, or inserted in the other two lineages. Note that the sequences from these three organisms, representative of the three domains of the living world, all differ from one another to a roughly similar degree, while still retaining unmistakable similarities.

Table 1–1 Some Genomes That Have Been Completely Sequenced

SPECIES	SPECIAL FEATURES	HABITAT	GENOME SIZE (1000s OF NUCLEOTIDE PAIRS PER HAPLOID GENOME)	ESTIMATED NUMBER OF GENES CODING FOR PROTEINS
BACTERIA				
Mycoplasma genitalium	has one of the smallest of all known cell genomes	human genital tract	580	468
Synechocystis sp.	photosynthetic, oxygen-generating (cyanobacterium)	lakes and streams	3573	3168
Escherichia coli	laboratory favorite	human gut	4639	4289
Helicobacter pylori	causes stomach ulcers and predisposes to stomach cancer	human stomach	1667	1590
Bacillus anthracis	causes anthrax	soil	5227	5634
Aquifex aeolicus	lithotrophic; lives at high temperatures	hydrothermal vents	1551	1544
Streptomyces coelicolor	source of antibiotics; giant genome	soil	8667	7825
Treponema pallidum	spirochete; causes syphilis	human tissues	1138	1041
Rickettsia prowazekii	bacterium most closely related to mitochondria; causes typhus	lice and humans (intracellular parasite)	1111	834
Thermotoga maritima	organotrophic; lives at very high temperatures	hydrothermal vents	1860	1877
ARCHAEA				
Methanococcus jannaschii	lithotrophic, anaerobic, methane-producing	hydrothermal vents	1664	1750
Archaeoglobus fulgidus	lithotrophic or organotrophic, anaerobic, sulfate-reducing	hydrothermal vents	2178	2493
Nanoarchaeum equitans	smallest known archaean; anaerobic; parasitic on another, larger archaean	hydrothermal and volcanic hot vents	491	552
EUCARYOTES				
Saccharomyces cerevisiae (budding yeast)	minimal model eucaryote	grape skins, beer	12,069	~6300
Arabidopsis thaliana (Thale cress)	model organism for flowering plants	soil and air	~142,000	~26,000
Caenorhabditis elegans (nematode worm)	simple animal with perfectly predictable development	soil	~97,000	~20,000
Drosophila melanogaster (fruit fly)	key to the genetics of animal development	rotting fruit	~137,000	~14,000
Homo sapiens (human)	most intensively studied mammal	houses	~3,200,000	~24,000

Genome size and gene number vary between strains of a single species, especially for bacteria and archaea. The table shows data for particular strains that have been sequenced. For eucaryotes, many genes can give rise to several alternative variant proteins, so that the total number of proteins specified by the genome is substantially greater than the number of genes.

New Genes Are Generated from Preexisting Genes

The raw material of evolution is the DNA sequence that already exists: there is no natural mechanism for making long stretches of new random sequence. In this sense, no gene is ever entirely new. Innovation can, however, occur in several ways (**Figure 1–23**):

1. *Intragenic mutation*: an existing gene can be modified by changes in its DNA sequence, through various types of error that occur mainly in the process of DNA replication.
2. *Gene duplication*: an existing gene can be duplicated so as to create a pair of initially identical genes within a single cell; these two genes may then diverge in the course of evolution.

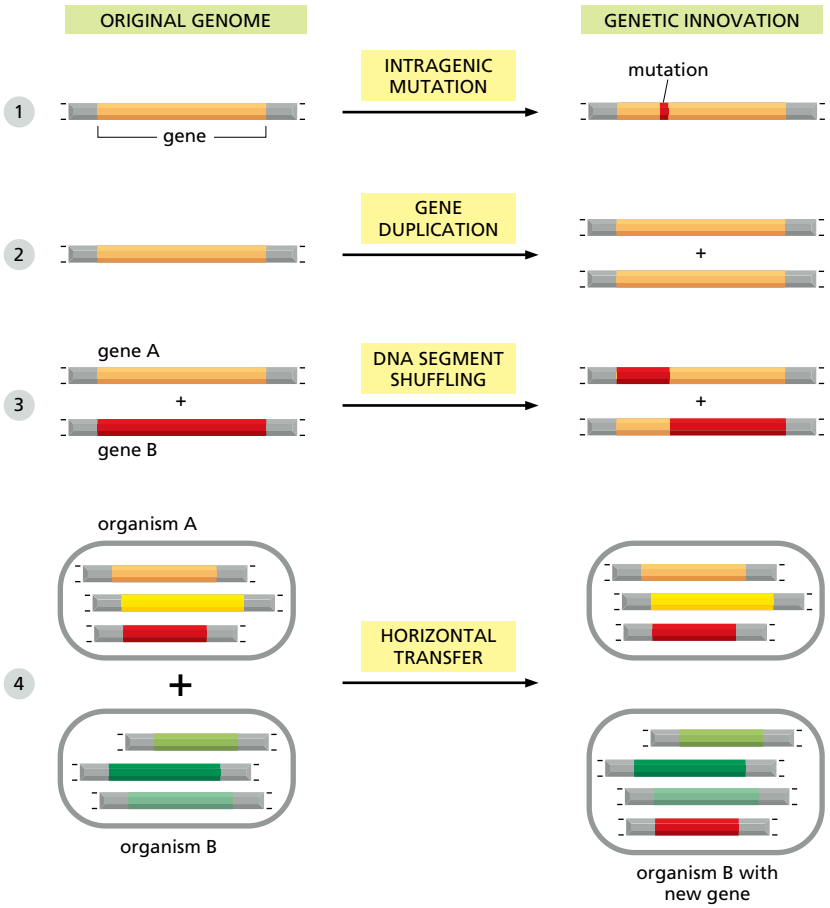


Figure 1–23 Four modes of genetic innovation and their effects on the DNA sequence of an organism. A special form of horizontal transfer occurs when two different types of cells enter into a permanent symbiotic association. Genes from one of the cells then may be transferred to the genome of the other, as we shall see below when we discuss mitochondria and chloroplasts.

3. *Segment shuffling*: two or more existing genes can be broken and rejoined to make a hybrid gene consisting of DNA segments that originally belonged to separate genes.
4. *Horizontal (intercellular) transfer*: a piece of DNA can be transferred from the genome of one cell to that of another—even to that of another species. This process is in contrast with the usual *vertical transfer* of genetic information from parent to progeny.

Each of these types of change leaves a characteristic trace in the DNA sequence of the organism, providing clear evidence that all four processes have occurred. In later chapters we discuss the underlying mechanisms, but for the present we focus on the consequences.

Gene Duplications Give Rise to Families of Related Genes Within a Single Cell

A cell duplicates its entire genome each time it divides into two daughter cells. However, accidents occasionally result in the inappropriate duplication of just part of the genome, with retention of original and duplicate segments in a single cell. Once a gene has been duplicated in this way, one of the two gene copies is free to mutate and become specialized to perform a different function within the same cell. Repeated rounds of this process of duplication and divergence, over many millions of years, have enabled one gene to give rise to a family of genes that may all be found within a single genome. Analysis of the DNA sequence of procaryotic genomes reveals many examples of such gene families: in *Bacillus subtilis*, for example, 47% of the genes have one or more obvious relatives (**Figure 1–24**).

When genes duplicate and diverge in this way, the individuals of one species become endowed with multiple variants of a primordial gene. This evolutionary

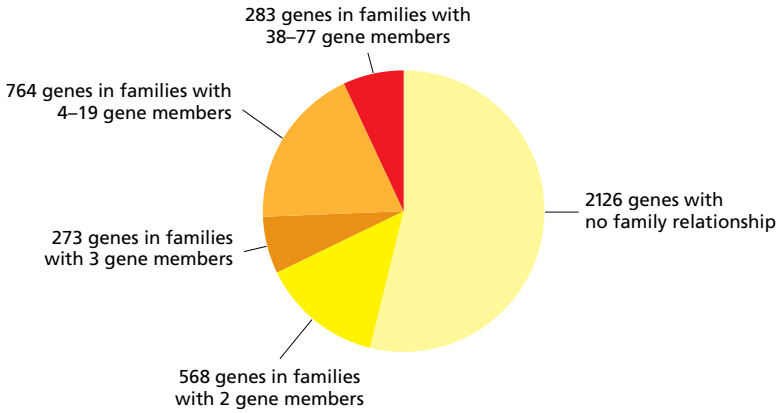


Figure 1–24 Families of evolutionarily related genes in the genome of *Bacillus subtilis*. The biggest family consists of 77 genes coding for varieties of ABC transporters—a class of membrane transport proteins found in all three domains of the living world. (Adapted from F. Kunst et al., *Nature* 390:249–256, 1997. With permission from Macmillan Publishers Ltd.)

process has to be distinguished from the genetic divergence that occurs when one species of organism splits into two separate lines of descent at a branch point in the family tree—when the human line of descent became separate from that of chimpanzees, for example. There, the genes gradually become different in the course of evolution, but they are likely to continue to have corresponding functions in the two sister species. Genes that are related by descent in this way—that is, genes in two separate species that derive from the same ancestral gene in the last common ancestor of those two species—are called **orthologs**. Related genes that have resulted from a gene duplication event within a single genome—and are likely to have diverged in their function—are called **paralogs**. Genes that are related by descent in either way are called **homologs**, a general term used to cover both types of relationship (**Figure 1–25**).

The family relationships between genes can become quite complex (**Figure 1–26**). For example, an organism that possesses a family of paralogous genes (for example, the seven hemoglobin genes α, β, γ, δ, ε, ζ, and θ) may evolve into two separate species (such as humans and chimpanzees) each possessing the entire set of paralogs. All 14 genes are homologs, with the human hemoglobin α orthologous to the chimpanzee hemoglobin α, but paralogous to the human or chimpanzee hemoglobin β, and so on. Moreover, the vertebrate hemoglobins (the oxygen-binding proteins of blood) are homologous to the vertebrate myoglobins (the oxygen-binding proteins of muscle), as well as to more distant

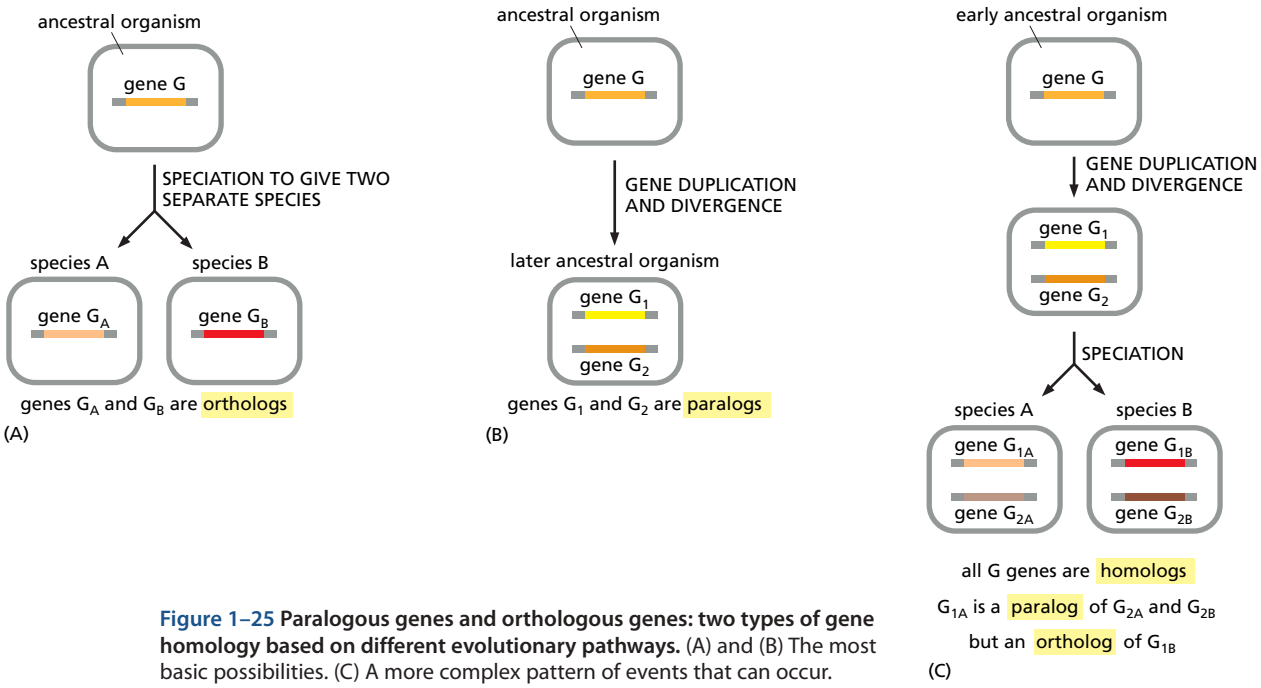


Figure 1–25 Paralogous genes and orthologous genes: two types of gene homology based on different evolutionary pathways. (A) and (B) The most basic possibilities. (C) A more complex pattern of events that can occur.

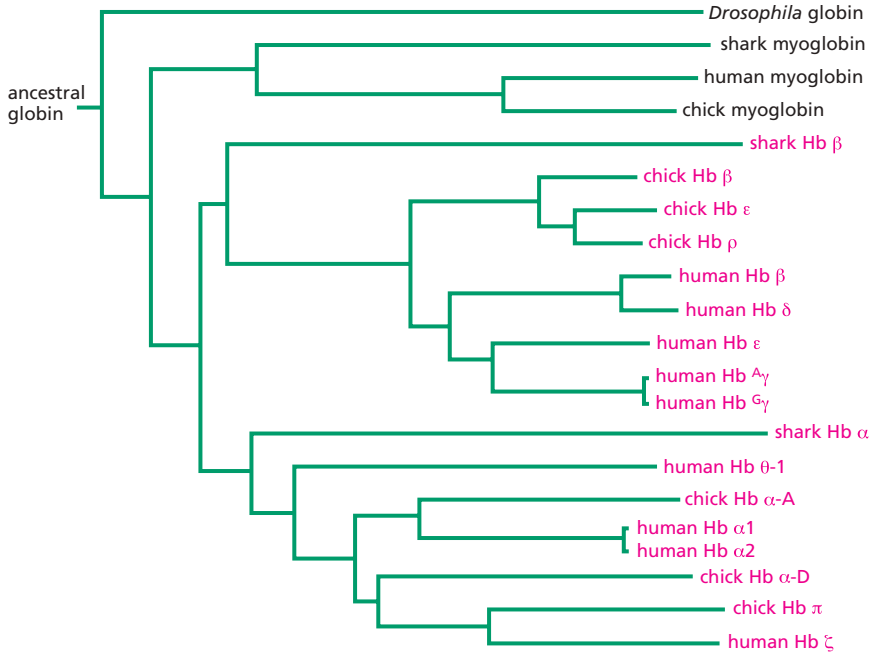


Figure 1–26 A complex family of homologous genes. This diagram shows the pedigree of the hemoglobin (Hb), myoglobin, and globin genes of human, chick, shark, and *Drosophila*. The lengths of the horizontal lines represent the amount of divergence in amino acid sequence.

genes that code for oxygen-binding proteins in invertebrates, plants, fungi, and bacteria. From the DNA sequences, it is usually easy to recognize that two genes in different species are homologous; it is much more difficult to decide, without other information, whether they stand in the precise evolutionary relationship of orthologs.

Genes Can Be Transferred Between Organisms, Both in the Laboratory and in Nature

Procaryotes also provide examples of the horizontal transfer of genes from one species of cell to another. The most obvious tell-tale signs are sequences recognizable as being derived from bacterial viruses, also called *bacteriophages* (**Figure 1–27**). **Viruses** are not themselves living cells but can act as vectors for gene transfer: they are small packets of genetic material that have evolved as parasites on the reproductive and biosynthetic machinery of host cells. They replicate in one cell, emerge from it with a protective wrapping, and then enter and infect another cell, which may be of the same or a different species. Often, the infected cell will be killed by the massive proliferation of virus particles inside it; but sometimes, the viral DNA, instead of directly generating these particles, may persist in its host for many cell generations as a relatively innocuous passenger, either as a separate intracellular fragment of DNA, known as a *plasmid,* or as a sequence inserted into the cell's regular genome. In their travels, viruses can accidentally pick up fragments of DNA from the genome of one host cell and ferry them into another cell. Such transfers of genetic material frequently occur in procaryotes, and they can also occur between eucaryotic cells of the same species.

Horizontal transfers of genes between eucaryotic cells of different species are very rare, and they do not seem to have played a significant part in eucaryote evolution (although massive transfers from bacterial to eucaryotic genomes have occurred in the evolution of mitochondria and chloroplasts, as we discuss below). In contrast, horizontal gene transfers occur much more frequently between different species of procaryotes. Many procaryotes have a remarkable capacity to take up even nonviral DNA molecules from their surroundings and thereby capture the genetic information these molecules carry. By this route, or by virus-mediated transfer, bacteria and archaea in the wild can acquire genes from neighboring cells relatively easily. Genes that confer resistance to an

antibiotic or an ability to produce a toxin, for example, can be transferred from species to species and provide the recipient bacterium with a selective advantage. In this way, new and sometimes dangerous strains of bacteria have been observed to evolve in the bacterial ecosystems that inhabit hospitals or the various niches in the human body. For example, horizontal gene transfer is responsible for the spread, over the past 40 years, of penicillin-resistant strains of *Neisseria gonorrheae,* the bacterium that causes gonorrhea. On a longer time scale, the results can be even more profound; it has been estimated that at least 18% of all of the genes in the present-day genome of *E. coli* have been acquired by horizontal transfer from another species within the past 100 million years.

Sex Results in Horizontal Exchanges of Genetic Information Within a Species

Horizontal exchanges of genetic information are important in bacterial and archaeal evolution in today's world, and they may have occurred even more frequently and promiscuously in the early days of life on Earth. Such early horizontal exchanges could explain the otherwise puzzling observation that the eucaryotes seem more similar to archaea in their genes for the basic information-handling processes of DNA replication, transcription, and translation, but more similar to bacteria in their genes for metabolic processes. In any case, whether horizontal gene transfer occurred most freely in the early days of life on Earth, or has continued at a steady low rate throughout evolutionary history, it has the effect of complicating the whole concept of cell ancestry, by making each cell's genome a composite of parts derived from separate sources.

Horizontal gene transfer among procaryotes may seem a surprising process, but it has a parallel in a phenomenon familiar to us all: sex. In addition to the usual vertical transfer of genetic material from parent to offspring, sexual reproduction causes a large-scale horizontal transfer of genetic information between two initially separate cell lineages—those of the father and the mother. A key feature of sex, of course, is that the genetic exchange normally occurs only between individuals of the same species. But no matter whether they occur within a species or between species, horizontal gene transfers leave a characteristic imprint: they result in individuals who are related more closely to one set of relatives with respect to some genes, and more closely to another set of relatives with respect to others. By comparing the DNA sequences of individual human genomes, an intelligent visitor from outer space could deduce that humans reproduce sexually, even if it knew nothing about human behavior.

Sexual reproduction is widespread (although not universal), especially among eucaryotes. Even bacteria indulge from time to time in controlled sexual exchanges of DNA with other members of their own species. Natural selection has clearly favored organisms that can reproduce sexually, although evolutionary theorists dispute precisely what the selective advantage of sex is.

The Function of a Gene Can Often Be Deduced from Its Sequence

Family relationships among genes are important not just for their historical interest, but because they simplify the task of deciphering gene functions. Once the sequence of a newly discovered gene has been determined, a scientist can tap a few keys on a computer to search the entire database of known gene sequences for genes related to it. In many cases, the function of one or more of these homologs will have been already determined experimentally, and thus, since gene sequence determines gene function, one can frequently make a good guess at the function of the new gene: it is likely to be similar to that of the already-known homologs.

In this way, it is possible to decipher a great deal of the biology of an organism simply by analyzing the DNA sequence of its genome and using the information we already have about the functions of genes in other organisms that have been more intensively studied.

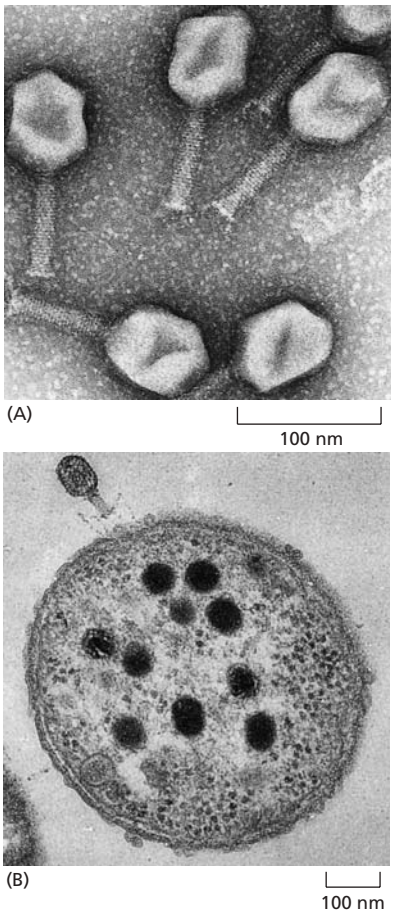


Figure 1–27 The viral transfer of DNA from one cell to another. (A) An electron micrograph of particles of a bacterial virus, the T4 bacteriophage. The head of this virus contains the viral DNA; the tail contains the apparatus for injecting the DNA into a host bacterium. (B) A cross section of a bacterium with a T4 bacteriophage latched onto its surface. The large dark objects inside the bacterium are the heads of new T4 particles in course of assembly. When they are mature, the bacterium will burst open to release them. (A, courtesy of James Paulson; B, courtesy of Jonathan King and Erika Hartwig from G. Karp, Cell and Molecular Biology, 2nd ed. New York: John Wiley & Sons, 1999. With permission from John Wiley & Sons.)

More Than 200 Gene Families Are Common to All Three Primary Branches of the Tree of Life

Given the complete genome sequences of representative organisms from all three domains—archaea, bacteria, and eucaryotes—we can search systematically for homologies that span this enormous evolutionary divide. In this way we can begin to take stock of the common inheritance of all living things. There are considerable difficulties in this enterprise. For example, individual species have often lost some of the ancestral genes; other genes have almost certainly been acquired by horizontal transfer from another species and therefore are not truly ancestral, even though shared. In fact, genome comparisons strongly suggest that both lineage-specific gene loss and horizontal gene transfer, in some cases between evolutionarily distant species, have been major factors of evolution, at least among procaryotes. Finally, in the course of 2 or 3 billion years, some genes that were initially shared will have changed beyond recognition by current methods.

Because of all these vagaries of the evolutionary process, it seems that only a small proportion of ancestral gene families have been universally retained in a recognizable form. Thus, out of 4873 protein-coding gene families defined by comparing the genomes of 50 species of bacteria, 13 archaea, and 3 unicellular eucaryotes, only 63 are truly ubiquitous (that is, represented in all the genomes analyzed). The great majority of these universal families include components of the translation and transcription systems. This is not likely to be a realistic approximation of an ancestral gene set. A better—though still crude—idea of the latter can be obtained by tallying the gene families that have representatives in multiple, but not necessarily all, species from all three major domains. Such an analysis reveals 264 ancient conserved families. Each family can be assigned a function (at least in terms of general biochemical activity, but usually with more precision), with the largest number of shared gene families being involved in translation and in amino acid metabolism and transport (**Table 1–2**). This set of highly conserved gene families represents only a very rough sketch of the common inheritance of all modern life; a more precise reconstruction of the gene complement of the last universal common ancestor might be feasible with further genome sequencing and more careful comparative analysis.

Mutations Reveal the Functions of Genes

Without additional information, no amount of gazing at genome sequences will reveal the functions of genes. We may recognize that gene B is like gene A, but how do we discover the function of gene A in the first place? And even if we know the function of gene A, how do we test whether the function of gene B is truly the same as the sequence similarity suggests? How do we connect the world of abstract genetic information with the world of real living organisms?

The analysis of gene functions depends on two complementary approaches: genetics and biochemistry. Genetics starts with the study of mutants: we either find or make an organism in which a gene is altered, and examine the effects on the organism's structure and performance (**Figure 1–28**). Biochemistry examines the functions of molecules: we extract molecules from an organism and then study their chemical activities. By combining genetics and biochemistry and examining the chemical abnormalities in a mutant organism, it is possible to find those molecules whose production depends on a given gene. At the same time, studies of the performance of the mutant organism show us what role those molecules have in the operation of the organism as a whole. Thus, genetics and biochemistry together provide a way to relate genes, molecules, and the structure and function of the organism.

In recent years, DNA sequence information and the powerful tools of molecular biology have allowed rapid progress. From sequence comparisons, we can often identify particular subregions within a gene that have been preserved nearly unchanged over the course of evolution. These conserved subregions are likely to be the most important parts of the gene in terms of function. We can test their individual contributions to the activity of the gene product by creating in

Table 1–2 The Numbers of Gene Families, Classified by Function, That Are Common to All Three Domains of the Living World

GENE FAMILY FUNCTION	NUMBER OF "UNIVERSAL" FAMILIES
Information processing	
Translation	63
Transcription	7
Replication, recombination, and repair	13
Cellular processes and signaling	
Cell cycle control, mitosis, and meiosis	2
Defense mechanisms	3
Signal transduction mechanisms	1
Cell wall/membrane biogenesis	2
Intracellular trafficking and secretion	4
Post-translational modification, protein turnover, chaperones	8
Metabolism	
Energy production and conversion	19
Carbohydrate transport and metabolism	16
Amino acid transport and metabolism	43
Nucleotide transport and metabolism	15
Coenzyme transport and metabolism	22
Lipid transport and metabolism	9
Inorganic ion transport and metabolism	8
Secondary metabolite biosynthesis, transport, and catabolism	5
Poorly characterized	
General biochemical function predicted; specific biological role unknown	24

For the purpose of this analysis, gene families are defined as "universal" if they are represented in the genomes of at least two diverse archaea *(Archaeoglobus fulgidus* and *Aeropyrum pernix),* two evolutionarily distant bacteria *(Escherichia coli* and *Bacillus subtilis)* and one eucaryote (yeast, *Saccharomyces cerevisiae).* (Data from R.L. Tatusov, E.V. Koonin and D.J. Lipman, *Science* 278:631–637, 1997, with permission from AAAS; R.L. Tatusov et al., *BMC Bioinformatics* 4:41, 2003, with permission from BioMed Central; and the COGs database at the US National Library of Medicine.)

the laboratory mutations of specific sites within the gene, or by constructing artificial hybrid genes that combine part of one gene with part of another. Organisms can be engineered to make either the RNA or the protein specified by the gene in large quantities to facilitate biochemical analysis. Specialists in molecular structure can determine the three-dimensional conformation of the gene product, revealing the exact position of every atom in it. Biochemists can determine how each of the parts of the genetically specified molecule contributes to its chemical behavior. Cell biologists can analyze the behavior of cells that are engineered to express a mutant version of the gene.

There is, however, no one simple recipe for discovering a gene's function, and no simple standard universal format for describing it. We may discover, for example, that the product of a given gene catalyzes a certain chemical reaction, and yet have no idea how or why that reaction is important to the organism. The functional characterization of each new family of gene products, unlike the description of the gene sequences, presents a fresh challenge to the biologist's ingenuity. Moreover, we never fully understand the function of a gene until we learn its role in the life of the organism as a whole. To make ultimate sense of gene functions, therefore, we have to study whole organisms, not just molecules or cells.

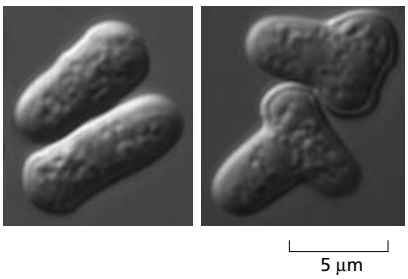


Figure 1–28 A mutant phenotype reflecting the function of a gene. A normal yeast (of the species *Schizosaccharomyces pombe)* is compared with a mutant in which a change in a single gene has converted the cell from a cigar shape *(left)* to a T shape *(right).* The mutant gene therefore has a function in the control of cell shape. But how, in molecular terms, does the gene product perform that function? That is a harder question, and needs biochemical analysis to answer it. (Courtesy of Kenneth Sawin and Paul Nurse.)

Molecular Biologists Have Focused a Spotlight on *E. coli*

Because living organisms are so complex, the more we learn about any particular species, the more attractive it becomes as an object for further study. Each

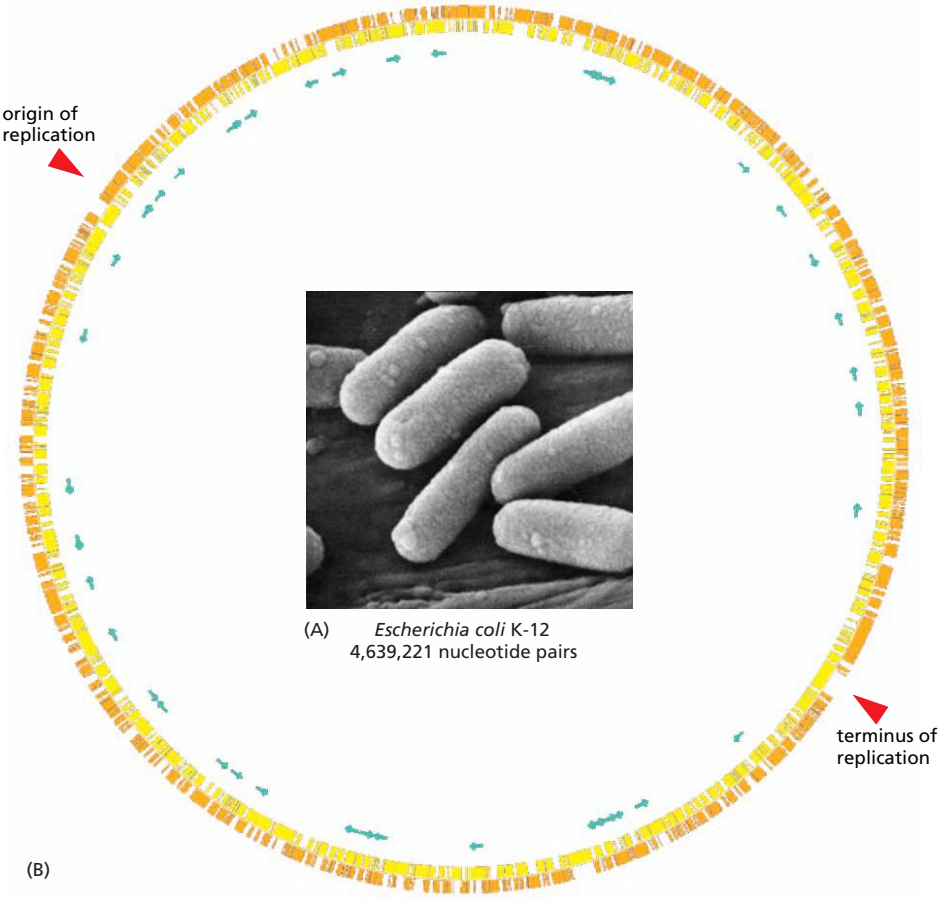


Figure 1–29 The genome of *E. coli*. (A) A cluster of *E. coli* cells. (B) A diagram of the genome of *E. coli* strain K-12. The diagram is circular because the DNA of *E. coli*, like that of other procaryotes, forms a single, closed loop. Protein-coding genes are shown as *yellow* or *orange bars*, depending on the DNA strand from which they are transcribed; genes encoding only RNA molecules are indicated by *green arrows*. Some genes are transcribed from one strand of the DNA double helix (in a clockwise direction in this diagram), others from the other strand (counterclockwise). (A, courtesy of Dr. Tony Brain and David Parker/Photo Researchers; B, adapted from F.R. Blattner et al., *Science* 277:1453–1462, 1997. With permission from AAAS.)

discovery raises new questions and provides new tools with which to tackle general questions in the context of the chosen organism. For this reason, large communities of biologists have become dedicated to studying different aspects of the same **model organism**.

In the enormously varied world of bacteria, the spotlight of molecular biology has for a long time focused intensely on just one species: *Escherichia coli*, or *E. coli* (see Figures 1–17 and 1–18). This small, rod-shaped bacterial cell normally lives in the gut of humans and other vertebrates, but it can be grown easily in a simple nutrient broth in a culture bottle. It adapts to variable chemical conditions and reproduces rapidly, and it can evolve by mutation and selection at a remarkable speed. As with other bacteria, different strains of *E. coli*, though classified as members of a single species, differ genetically to a much greater degree than do different varieties of a sexually reproducing organism such as a plant or animal. One *E. coli* strain may possess many hundreds of genes that are absent from another, and the two strains could have as little as 50% of their genes in common. The standard laboratory strain *E. coli* K-12 has a genome of approximately 4.6 million nucleotide pairs, contained in a single circular molecule of DNA, coding for about 4300 different kinds of proteins (**Figure 1–29**).

In molecular terms, we know more about *E. coli* than about any other living organism. Most of our understanding of the fundamental mechanisms of life—for example, how cells replicate their DNA, or how they decode the instructions represented in the DNA to direct the synthesis of specific proteins—has come from studies of *E. coli*. The basic genetic mechanisms have turned out to be highly conserved throughout evolution: these mechanisms are therefore essentially the same in our own cells as in *E. coli*.

Summary

Procaryotes (cells without a distinct nucleus) are biochemically the most diverse organisms and include species that can obtain all their energy and nutrients from inorganic chemical sources, such as the reactive mixtures of minerals released at hydrothermal vents on the ocean floor—the sort of diet that may have nourished the first living cells 3.5 billion years ago. DNA sequence comparisons reveal the family relationships of living organisms and show that the procaryotes fall into two groups that diverged early in the course of evolution: the bacteria (or eubacteria) and the archaea. Together with the eucaryotes (cells with a membrane-enclosed nucleus), these constitute the three primary branches of the tree of life. Most bacteria and archaea are small unicellular organisms with compact genomes comprising 1000–6000 genes. Many of the genes within a single organism show strong family resemblances in their DNA sequences, implying that they originated from the same ancestral gene through gene duplication and divergence. Family resemblances (homologies) are also clear when gene sequences are compared between different species, and more than 200 gene families have been so highly conserved that they can be recognized as common to most species from all three domains of the living world. Thus, given the DNA sequence of a newly discovered gene, it is often possible to deduce the gene's function from the known function of a homologous gene in an intensively studied model organism, such as the bacterium E. coli.

GENETIC INFORMATION IN EUCARYOTES

Eucaryotic cells, in general, are bigger and more elaborate than procaryotic cells, and their genomes are bigger and more elaborate, too. The greater size is accompanied by radical differences in cell structure and function. Moreover, many classes of eucaryotic cells form multicellular organisms that attain levels of complexity unmatched by any procaryote.

Because they are so complex, eucaryotes confront molecular biologists with a special set of challenges, which will concern us in the rest of this book. Increasingly, biologists meet these challenges through the analysis and manipulation of the genetic information within cells and organisms. It is therefore important at the outset to know something of the special features of the eucaryotic genome. We begin by briefly discussing how eucaryotic cells are organized, how this reflects their way of life, and how their genomes differ from those of procaryotes. This leads us to an outline of the strategy by which molecular biologists, by exploiting genetic information, are attempting to discover how eucaryotic organisms work.

Eucaryotic Cells May Have Originated as Predators

By definition, eucaryotic cells keep their DNA in an internal compartment called the nucleus. The *nuclear envelope,* a double layer of membrane, surrounds the nucleus and separates the DNA from the cytoplasm. Eucaryotes also have other features that set them apart from procaryotes (**Figure 1–30**). Their cells are, typically, 10 times bigger in linear dimension, and 1000 times larger in volume. They have a *cytoskeleton*—a system of protein filaments crisscrossing the cytoplasm and forming, together with the many proteins that attach to them, a system of girders, ropes, and motors that gives the cell mechanical strength, controls its shape, and drives and guides its movements. <GTTA> <ATGG> <TCGC> The nuclear envelope is only one part of a set of *internal membranes*, each structurally similar to the plasma membrane and enclosing different types of spaces inside the cell, many of them involved in digestion and secretion. Lacking the tough cell wall of most bacteria, animal cells and the free-living eucaryotic cells called *protozoa* can change their shape rapidly and engulf other cells and small objects by *phagocytosis* (**Figure 1–31**).

It is still a mystery how all these properties evolved, and in what sequence. One plausible view, however, is that they are all reflections of the way of life of a

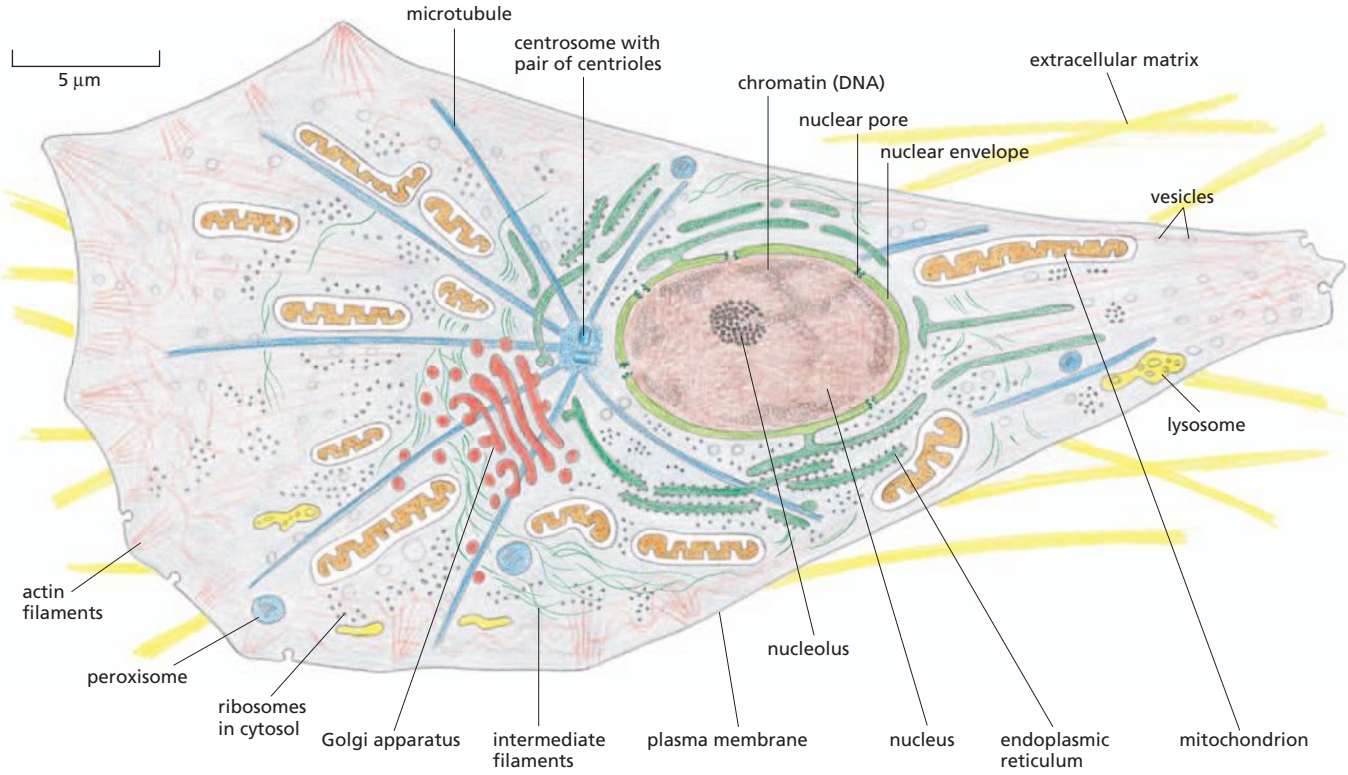


Figure 1–30 The major features of eucaryotic cells. The drawing depicts a typical animal cell, but almost all the same components are found in plants and fungi and in single-celled eucaryotes such as yeasts and protozoa. Plant cells contain chloroplasts in addition to the components shown here, and their plasma membrane is surrounded by a tough external wall formed of cellulose.

primordial eucaryotic cell that was a predator, living by capturing other cells and eating them (**Figure 1–32**). Such a way of life requires a large cell with a flexible plasma membrane, as well as an elaborate cytoskeleton to support and move this membrane. It may also require that the cell's long, fragile DNA molecules be sequestered in a separate nuclear compartment, to protect the genome from damage by the movements of the cytoskeleton.

Modern Eucaryotic Cells Evolved from a Symbiosis

A predatory way of life helps to explain another feature of eucaryotic cells. Almost all such cells contain *mitochondria* (**Figure 1–33**). These small bodies in the cytoplasm, enclosed by a double layer of membrane, take up oxygen and harness energy from the oxidation of food molecules—such as sugars—to produce most of the ATP that powers the cell's activities. Mitochondria are similar in size to small bacteria, and, like bacteria, they have their own genome in the form of a circular DNA molecule, their own ribosomes that differ from those elsewhere in the eucaryotic cell, and their own transfer RNAs. It is now generally accepted that mitochondria originated from free-living oxygen-metabolizing *(aerobic)* bacteria that were engulfed by an ancestral eucaryotic cell that could otherwise make no such use of oxygen (that is, was *anaerobic)*. Escaping digestion, these bacteria evolved in symbiosis with the engulfing cell and its progeny,

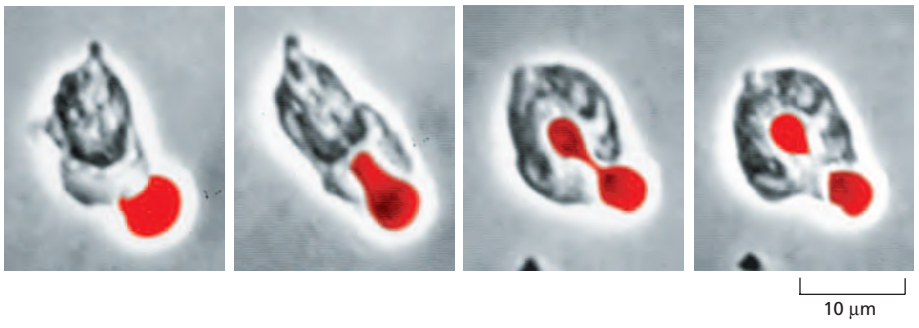


Figure 1–31 Phagocytosis. This series of stills from a movie shows a human white blood cell (a neutrophil) engulfing a red blood cell (artificially colored *red)* that has been treated with antibody. (Courtesy of Stephen E. Malawista and Anne de Boisfleury Chevance.)

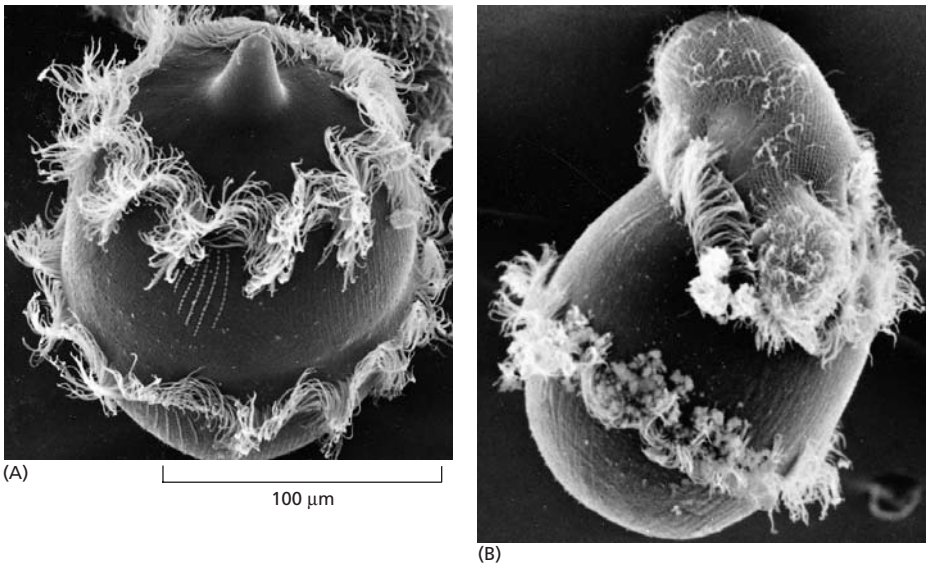


Figure 1–32 A single-celled eucaryote that eats other cells. (A) *Didinium* is a carnivorous protozoan, belonging to the group known as *ciliates*. It has a globular body, about 150 μm in diameter, encircled by two fringes of cilia—sinuous, whiplike appendages that beat continually; its front end is flattened except for a single protrusion, rather like a snout. (B) *Didinium* normally swims around in the water at high speed by means of the synchronous beating of its cilia. When it encounters a suitable prey, usually another type of protozoan, it releases numerous small paralyzing darts from its snout region. Then, the *Didinium* attaches to and devours the other cell by phagocytosis, inverting like a hollow ball to engulf its victim, which is almost as large as itself. (Courtesy of D. Barlow.)

receiving shelter and nourishment in return for the power generation they performed for their hosts (**Figure 1–34**). This partnership between a primitive anaerobic eucaryotic predator cell and an aerobic bacterial cell is thought to have been established about 1.5 billion years ago, when the Earth's atmosphere first became rich in oxygen.

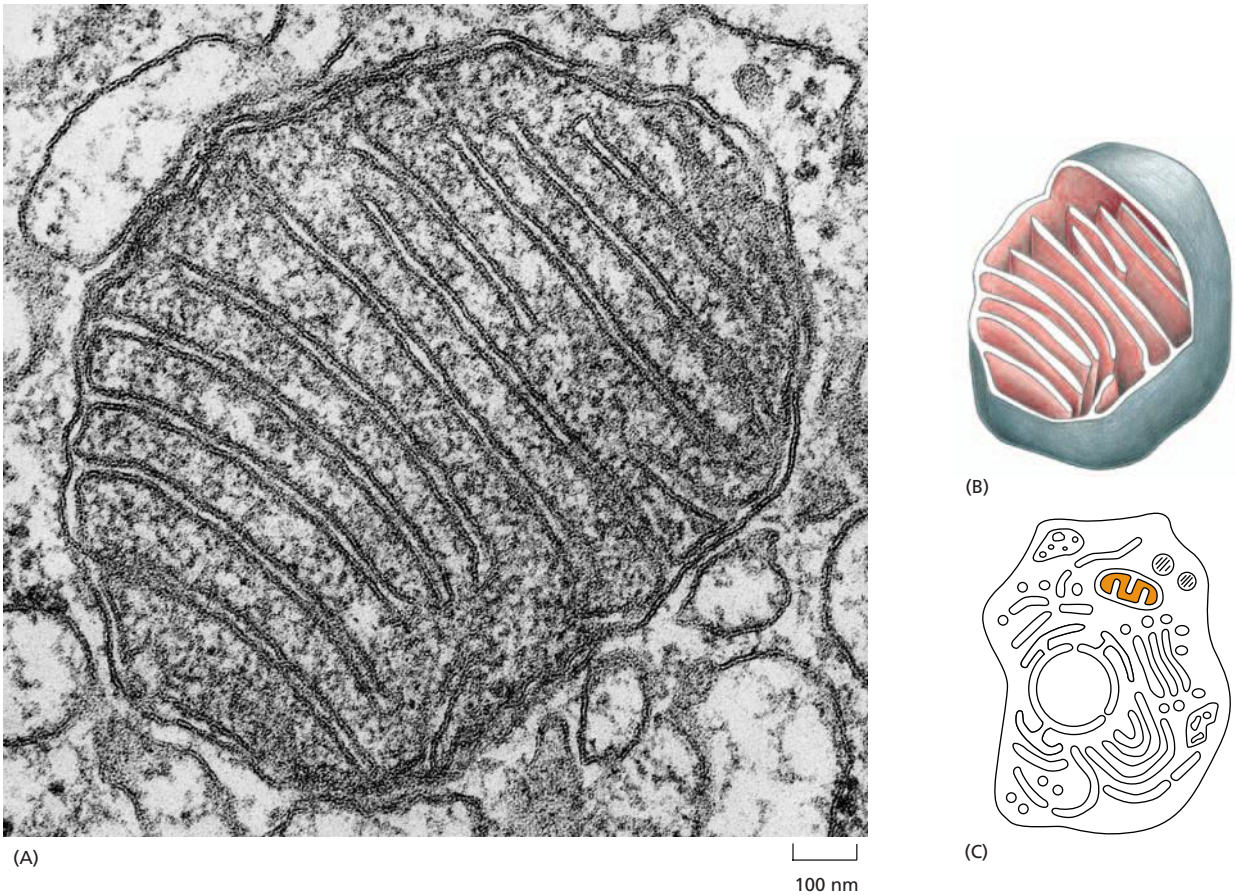


Figure 1–33 A mitochondrion. (A) A cross section, as seen in the electron microscope. (B) A drawing of a mitochondrion with part of it cut away to show the three-dimensional structure. (C) A schematic eucaryotic cell, with the interior space of a mitochondrion, containing the mitochondrial DNA and ribosomes, colored. Note the smooth outer membrane and the convoluted inner membrane, which houses the proteins that generate ATP from the oxidation of food molecules. (A, courtesy of Daniel S. Friend.)

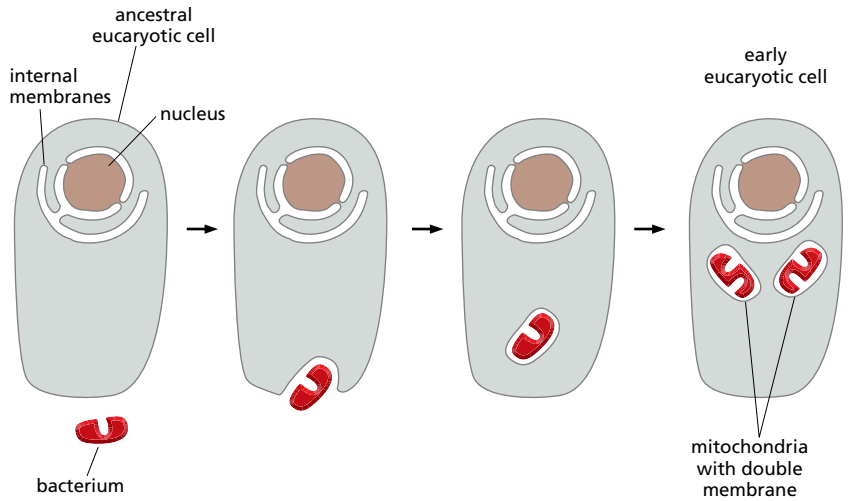


Figure 1–34 The origin of mitochondria. An ancestral eucaryotic cell is thought to have engulfed the bacterial ancestor of mitochondria, initiating a symbiotic relationship.

Many eucaryotic cells—specifically, those of plants and algae—also contain another class of small membrane-enclosed organelles somewhat similar to mitochondria—the *chloroplasts* (**Figure 1–35**). Chloroplasts perform photosynthesis, using the energy of sunlight to synthesize carbohydrates from atmospheric carbon dioxide and water, and deliver the products to the host cell as food. Like mitochondria, chloroplasts have their own genome and almost certainly originated as symbiotic photosynthetic bacteria, acquired by cells that already possessed mitochondria (**Figure 1–36**).

A eucaryotic cell equipped with chloroplasts has no need to chase after other cells as prey; it is nourished by the captive chloroplasts it has inherited from its ancestors. Correspondingly, plant cells, although they possess the cytoskeletal equipment for movement, have lost the ability to change shape rapidly and to engulf other cells by phagocytosis. Instead, they create around themselves a tough, protective cell wall. If the ancestral eucaryote was indeed a predator on other organisms, we can view plant cells as eucaryotes that have made the transition from hunting to farming.

Fungi represent yet another eucaryotic way of life. Fungal cells, like animal cells, possess mitochondria but not chloroplasts; but in contrast with animal cells and protozoa, they have a tough outer wall that limits their ability to move

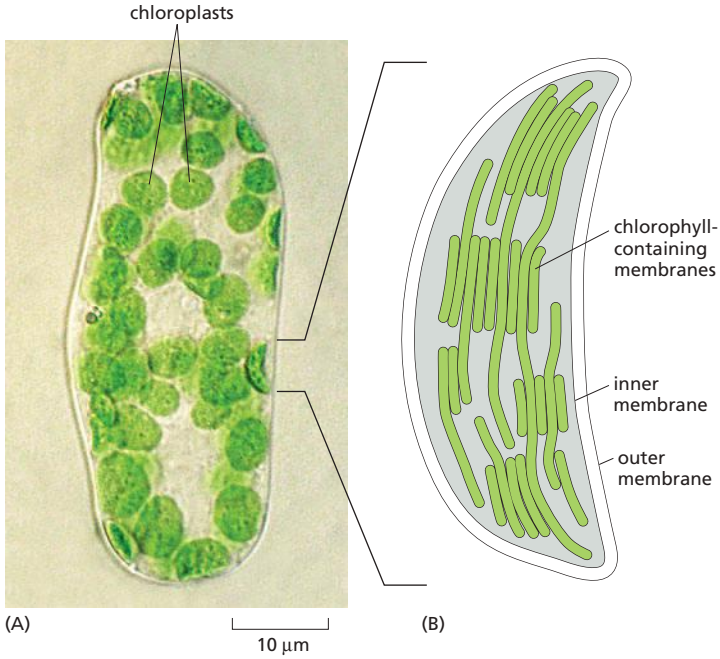


Figure 1–35 Chloroplasts. These organelles capture the energy of sunlight in plant cells and some single-celled eucaryotes. (A) A single cell isolated from a leaf of a flowering plant, seen in the light microscope, showing the green chloroplasts. (B) A drawing of one of the chloroplasts, showing the highly folded system of internal membranes containing the chlorophyll molecules by which light is absorbed. (A, courtesy of Preeti Dahiya.)

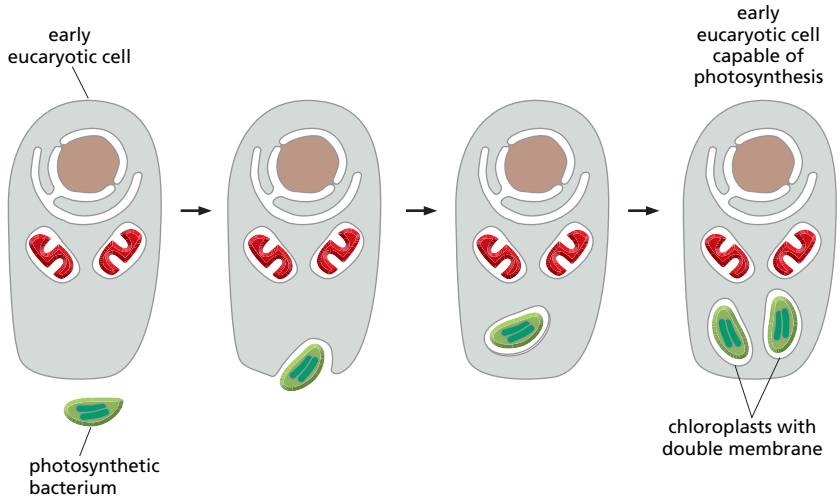


Figure 1–36 The origin of chloroplasts. An early eucaryotic cell, already possessing mitochondria, engulfed a photosynthetic bacterium (a cyanobacterium) and retained it in symbiosis. All present-day chloroplasts are thought to trace their ancestry back to a single species of cyanobacterium that was adopted as an internal symbiont (an endosymbiont) over a billion years ago.

rapidly or to swallow up other cells. Fungi, it seems, have turned from hunters into scavengers: other cells secrete nutrient molecules or release them upon death, and fungi feed on these leavings—performing whatever digestion is necessary extracellularly, by secreting digestive enzymes to the exterior.

Eucaryotes Have Hybrid Genomes

The genetic information of eucaryotic cells has a hybrid origin—from the ancestral anaerobic eucaryote, and from the bacteria that it adopted as symbionts. Most of this information is stored in the nucleus, but a small amount remains inside the mitochondria and, for plant and algal cells, in the chloroplasts. The mitochondrial DNA and the chloroplast DNA can be separated from the nuclear DNA and individually analyzed and sequenced. The mitochondrial and chloroplast genomes are found to be degenerate, cut-down versions of the corresponding bacterial genomes, lacking genes for many essential functions. In a human cell, for example, the mitochondrial genome consists of only 16,569 nucleotide pairs, and codes for only 13 proteins, two ribosomal RNA components, and 22 transfer RNAs.

The genes that are missing from the mitochondria and chloroplasts have not all been lost; instead, many of them have been somehow moved from the symbiont genome into the DNA of the host cell nucleus. The nuclear DNA of humans contains many genes coding for proteins that serve essential functions inside the mitochondria; in plants, the nuclear DNA also contains many genes specifying proteins required in chloroplasts.

Eucaryotic Genomes Are Big

Natural selection has evidently favored mitochondria with small genomes, just as it has favored bacteria with small genomes. By contrast, the nuclear genomes of most eucaryotes seem to have been free to enlarge. Perhaps the eucaryotic way of life has made large size an advantage: predators typically need to be bigger than their prey, and cell size generally increases in proportion to genome size. Perhaps enlargement of the genome has been driven by the accumulation of parasitic transposable elements (discussed in Chapter 5)—"selfish" segments of DNA that can insert copies of themselves at multiple sites in the genome. Whatever the explanation, the genomes of most eucaryotes are orders of magnitude larger than those of bacteria and archaea (**Figure 1–37**). And the freedom to be extravagant with DNA has had profound implications.

Eucaryotes not only have more genes than procaryotes; they also have vastly more DNA that does not code for protein or for any other functional product molecule. The human genome contains 1000 times as many nucleotide pairs as the genome of a typical bacterium, 20 times as many genes, and about 10,000

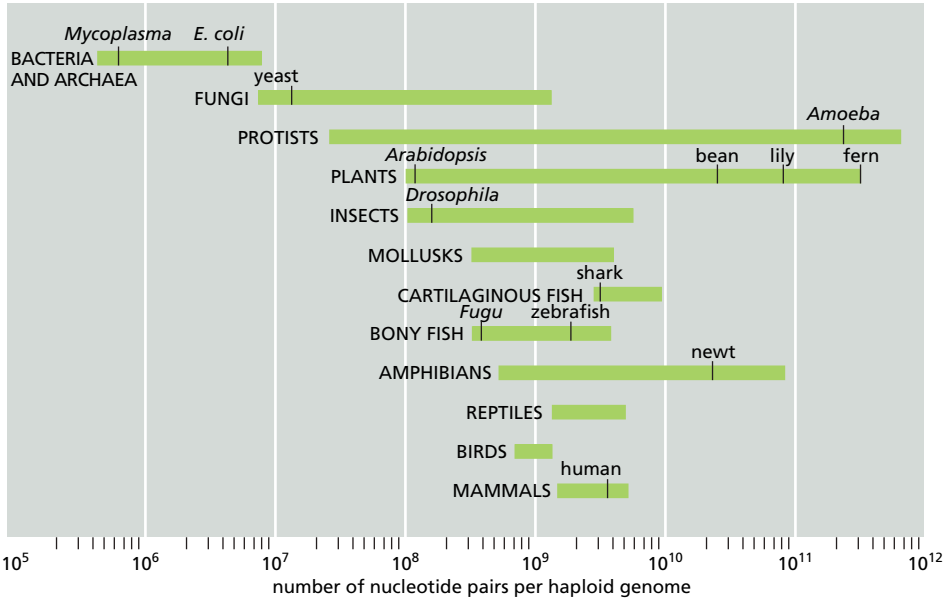


Figure 1–37 Genome sizes compared. Genome size is measured in nucleotide pairs of DNA per haploid genome, that is, per single copy of the genome. (The cells of sexually reproducing organisms such as ourselves are generally diploid: they contain two copies of the genome, one inherited from the mother, the other from the father.) Closely related organisms can vary widely in the quantity of DNA in their genomes, even though they contain similar numbers of functionally distinct genes. (Data from W.H. Li, Molecular Evolution, pp. 380–383. Sunderland, MA: Sinauer, 1997.)

times as much noncoding DNA (~98.5% of the genome for a human is noncoding, as opposed to 11% of the genome for the bacterium *E. coli*).

Eucaryotic Genomes Are Rich in Regulatory DNA

Much of our noncoding DNA is almost certainly dispensable junk, retained like a mass of old papers because, when there is little pressure to keep an archive small, it is easier to retain everything than to sort out the valuable information and discard the rest. Certain exceptional eucaryotic species, such as the puffer fish (**Figure 1–38**), bear witness to the profligacy of their relatives; they have somehow managed to rid themselves of large quantities of noncoding DNA. Yet they appear similar in structure, behavior, and fitness to related species that have vastly more such DNA.

Even in compact eucaryotic genomes such as that of puffer fish, there is more noncoding DNA than coding DNA, and at least some of the noncoding DNA certainly has important functions. In particular, it regulates the expression of adjacent genes. With this regulatory DNA, eucaryotes have evolved distinctive ways of controlling when and where a gene is brought into play. This sophisticated gene regulation is crucial for the formation of complex multicellular organisms.

The Genome Defines the Program of Multicellular Development

The cells in an individual animal or plant are extraordinarily varied. Fat cells, skin cells, bone cells, nerve cells—they seem as dissimilar as any cells could be. Yet all these cell types are the descendants of a single fertilized egg cell, and all (with minor exceptions) contain identical copies of the genome of the species.

The differences result from the way in which the cells make selective use of their genetic instructions according to the cues they get from their surroundings in the developing embryo. The DNA is not just a shopping list specifying the molecules that every cell must have, and the cell is not an assembly of all the items on the list. Rather, the cell behaves as a multipurpose machine, with sensors to receive environmental signals and with highly developed abilities to call different sets of genes into action according to the sequences of signals to which the cell has been exposed. The genome in each cell is big enough to accommodate the information that specifies an entire multicellular organism, but in any individual cell only part of that information is used.

A large fraction of the genes in the eucaryotic genome code for proteins that regulate the activities of other genes. Most of these *gene regulatory proteins* act by



Figure 1–38 The puffer fish *(Fugu rubripes).* This organism has a genome size of 400 million nucleotide pairs—about one-quarter as much as a zebrafish, for example, even though the two species of fish have similar numbers of genes. (From a woodcut by Hiroshige, courtesy of Arts and Designs of Japan.)

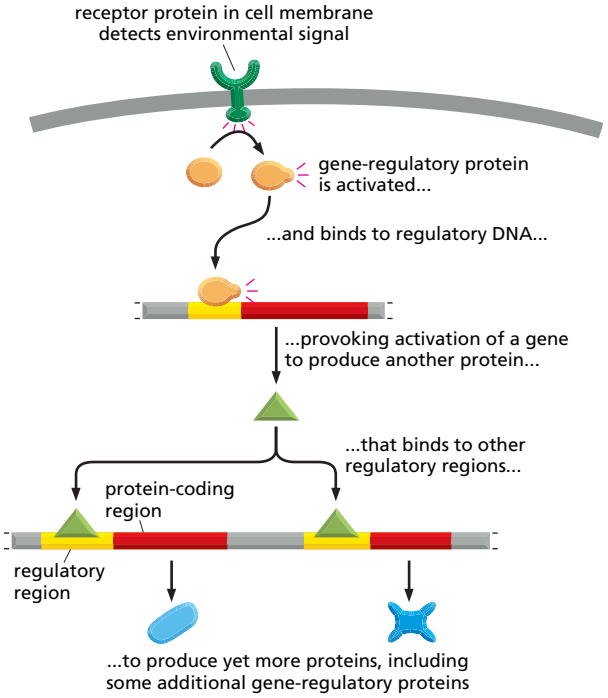


Figure 1–39 Controlling gene readout by environmental signals. Regulatory DNA allows gene expression to be controlled by regulatory proteins, which are in turn the products of other genes. This diagram shows how a cell's gene expression is adjusted according to a signal from the cell's environment. The initial effect of the signal is to activate a regulatory protein already present in the cell; the signal may, for example, trigger the attachment of a phosphate group to the regulatory protein, altering its chemical properties.

binding, directly or indirectly, to the regulatory DNA adjacent to the genes that are to be controlled (**Figure 1–39**), or by interfering with the abilities of other proteins to do so. The expanded genome of eucaryotes therefore not only specifies the hardware of the cell, but also stores the software that controls how that hardware is used (**Figure 1–40**).

Cells do not just passively receive signals; rather, they actively exchange signals with their neighbors. Thus, in a developing multicellular organism, the same control system governs each cell, but with different consequences depending on the messages exchanged. The outcome, astonishingly, is a precisely patterned array of cells in different states, each displaying a character appropriate to its position in the multicellular structure.

Many Eucaryotes Live as Solitary Cells: the Protists

Many species of eucaryotic cells lead a solitary life—some as hunters (the *protozoa*), some as photosynthesizers (the unicellular *algae*), some as scavengers (the unicellular fungi, or *yeasts*). **Figure 1–41** conveys something of the variety of forms of these single-celled eucaryotes, or *protists*. The anatomy of protozoa,



Figure 1–40 Genetic control of the program of multicellular development. The role of a regulatory gene is demonstrated in the snapdragon *Antirrhinum*. In this example, a mutation in a single gene coding for a regulatory protein causes leafy shoots to develop in place of flowers: because a regulatory protein has been changed, the cells adopt characters that would be appropriate to a different location in the normal plant. The mutant is on the left, the normal plant on the right. (Courtesy of Enrico Coen and Rosemary Carpenter.)

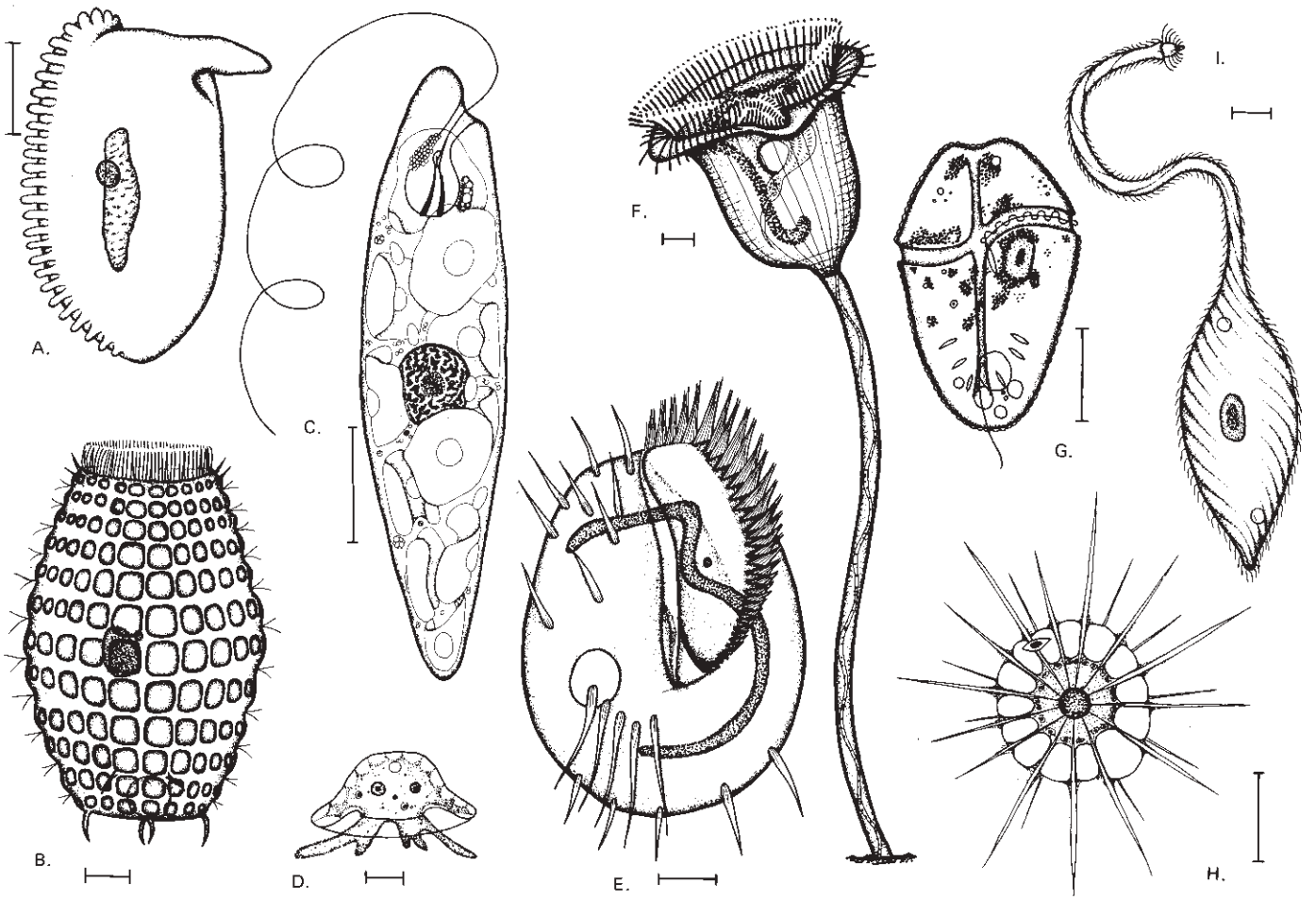


Figure 1–41 An assortment of protists: a small sample of an extremely diverse class of organisms. The drawings are done to different scales, but in each case the scale bar represents 10 μm. The organisms in (A), (B), (E), (F), and (I) are ciliates; (C) is a euglenoid; (D) is an amoeba; (G) is a dinoflagellate; (H) is a heliozoan. (From M.A. Sleigh, Biology of Protozoa. Cambridge, UK: Cambridge University Press, 1973.)

especially, is often elaborate and includes such structures as sensory bristles, photoreceptors, sinuously beating cilia, leglike appendages, mouth parts, stinging darts, and musclelike contractile bundles. Although they are single cells, protozoa can be as intricate, as versatile, and as complex in their behavior as many multicellular organisms (see Figure 1–32). <ATGG> <TCGC>

In terms of their ancestry and DNA sequences, protists are far more diverse than the multicellular animals, plants, and fungi, which arose as three comparatively late branches of the eucaryotic pedigree (see Figure 1–21). As with procaryotes, humans have tended to neglect the protists because they are microscopic. Only now, with the help of genome analysis, are we beginning to understand their positions in the tree of life, and to put into context the glimpses these strange creatures offer us of our distant evolutionary past.

A Yeast Serves as a Minimal Model Eucaryote

The molecular and genetic complexity of eucaryotes is daunting. Even more than for procaryotes, biologists need to concentrate their limited resources on a few selected model organisms to fathom this complexity.

To analyze the internal workings of the eucaryotic cell, without the additional problems of multicellular development, it makes sense to use a species that is unicellular and as simple as possible. The popular choice for this role of minimal model eucaryote has been the yeast *Saccharomyces cerevisiae* (**Figure 1–42**)—the same species that is used by brewers of beer and bakers of bread.

S. cerevisiae is a small, single-celled member of the kingdom of fungi and thus, according to modern views, at least as closely related to animals as it is to plants. It is robust and easy to grow in a simple nutrient medium. Like other fungi, it has a tough cell wall, is relatively immobile, and possesses mitochondria but not chloroplasts. When nutrients are plentiful, it grows and divides almost as

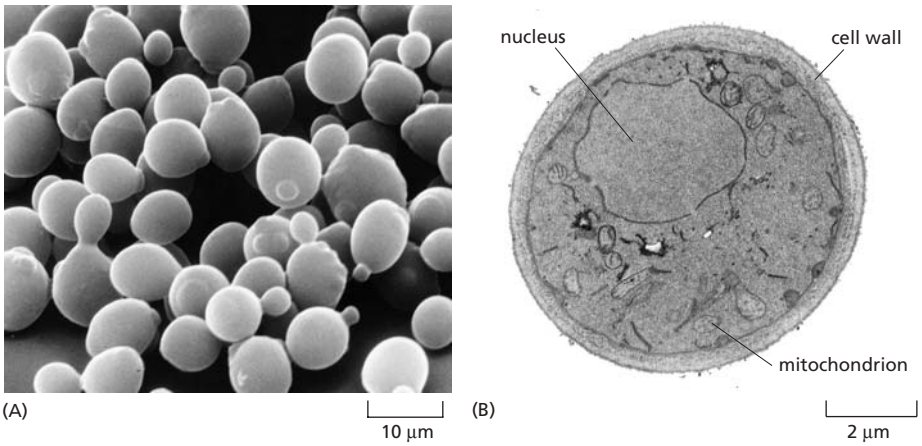


Figure 1–42 The yeast *Saccharomyces cerevisiae*. (A) A scanning electron micrograph of a cluster of the cells. This species is also known as budding yeast; it proliferates by forming a protrusion or bud that enlarges and then separates from the rest of the original cell. Many cells with buds are visible in this micrograph. (B) A transmission electron micrograph of a cross section of a yeast cell, showing its nucleus, mitochondrion, and thick cell wall. (A, courtesy of Ira Herskowitz and Eric Schabatach.)

rapidly as a bacterium. It can reproduce either vegetatively (that is, by simple cell division), or sexually: two yeast cells that are *haploid* (possessing a single copy of the genome) can fuse to create a cell that is *diploid* (containing a double genome); and the diploid cell can undergo *meiosis* (a reduction division) to produce cells that are once again haploid (**Figure 1–43**). In contrast with higher plants and animals, the yeast can divide indefinitely in either the haploid or the diploid state, and the process leading from the one state to the other can be induced at will by changing the growth conditions.

In addition to these features, the yeast has a further property that makes it a convenient organism for genetic studies: its genome, by eucaryotic standards, is exceptionally small. Nevertheless, it suffices for all the basic tasks that every eucaryotic cell must perform. As we shall see later in this book, studies on yeasts (using both *S. cerevisiae* and other species) have provided a key to many crucial processes, including the eucaryotic cell-division cycle—the critical chain of events by which the nucleus and all the other components of a cell are duplicated and parceled out to create two daughter cells from one. The control system that governs this process has been so well conserved over the course of evolution that many of its components can function interchangeably in yeast and human cells: if a mutant yeast lacking an essential yeast cell-division-cycle gene is supplied with a copy of the homologous cell-division-cycle gene from a human, the yeast is cured of its defect and becomes able to divide normally.

The Expression Levels of All The Genes of An Organism Can Be Monitored Simultaneously

The complete genome sequence of *S. cerevisiae*, determined in 1997, consists of approximately 13,117,000 nucleotide pairs, including the small contribution (78,520 nucleotide pairs) of the mitochondrial DNA. This total is only about 2.5 times as much DNA as there is in *E. coli*, and it codes for only 1.5 times as many distinct proteins (about 6300 in all). The way of life of *S. cerevisiae* is similar in many ways to that of a bacterium, and it seems that this yeast has likewise been subject to selection pressures that have kept its genome compact.

Knowledge of the complete genome sequence of any organism—be it a yeast or a human—opens up new perspectives on the workings of the cell: things that once seemed impossibly complex now seem within our grasp. Using techniques

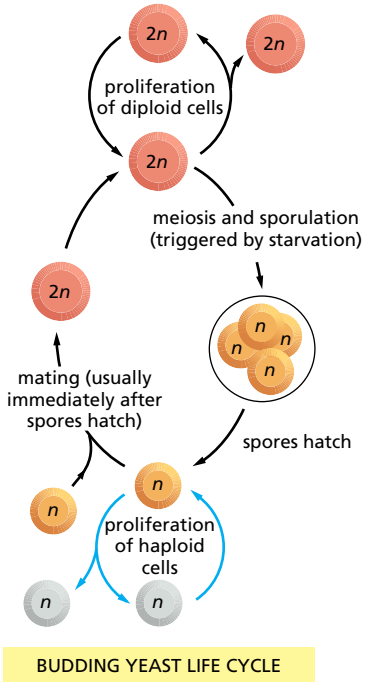


Figure 1–43 The reproductive cycles of the yeast *S. cerevisiae*. Depending on environmental conditions and on details of the genotype, cells of this species can exist in either a diploid (2*n*) state, with a double chromosome set, or a haploid (*n*) state, with a single chromosome set. The diploid form can either proliferate by ordinary cell-division cycles or undergo meiosis to produce haploid cells. The haploid form can either proliferate by ordinary cell-division cycles or undergo sexual fusion with another haploid cell to become diploid. Meiosis is triggered by starvation and gives rise to spores—haploid cells in a dormant state, resistant to harsh environmental conditions.

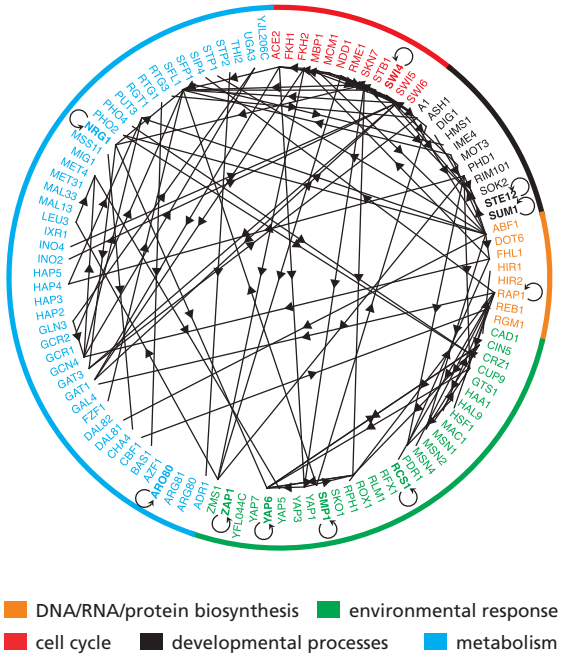


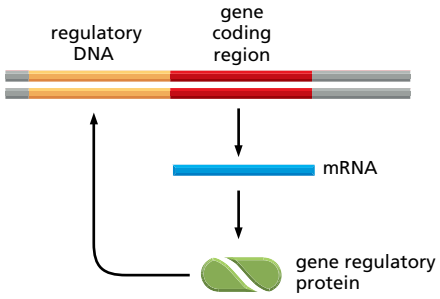
Figure 1–44 The network of interactions between gene regulatory proteins and the genes that code for them in a yeast cell. Results are shown for 106 out of the total of 141 gene regulatory proteins in *Saccharomyces cerevisiae*. Each protein in the set was tested for its ability to bind to the regulatory DNA of each of the genes coding for this set of proteins. In the diagram, the genes are arranged in a circle, and an arrow pointing from gene A to gene B means that the protein encoded by A binds to the regulatory DNA of B, and therefore presumably regulates the expression of B. Small circles with arrowheads indicate genes whose products directly regulate their own expression. Genes governing different aspects of cell behavior are shown in different colors. For a multicellular plant or animal, the number of gene regulatory proteins is about 10 times greater, and the amount of regulatory DNA perhaps 100 times greater, so that the corresponding diagram would be vastly more complex. (From T.I. Lee et al., *Science* 298:799–804, 2002. With permission from AAAS.)

to be described in Chapter 8, it is now possible, for example, to monitor, simultaneously, the amount of mRNA transcript that is produced from every gene in the yeast genome under any chosen conditions, and to see how this whole pattern of gene activity changes when conditions change. The analysis can be repeated with mRNA prepared from mutants lacking a chosen gene—any gene that we care to test. In principle, this approach provides a way to reveal the entire system of control relationships that govern gene expression—not only in yeast cells, but in any organism whose genome sequence is known.

To Make Sense of Cells, We Need Mathematics, Computers, and Quantitative Information

Through methods such as these, exploiting our knowledge of complete genome sequences, we can list the genes and proteins in a cell and begin to depict the web of interactions between them (**Figure 1–44**). But how are we to turn all this information into an understanding of how cells work? Even for a single cell type belonging to a single species of organism, the current deluge of data seems overwhelming. The sort of informal reasoning on which biologists usually rely seems totally inadequate in the face of such complexity. In fact, the difficulty is more than just a matter of information overload. Biological systems are, for example, full of feedback loops, and the behavior of even the simplest of systems with feedback is remarkably difficult to predict by intuition alone (**Figure 1–45**); small

Figure 1–45 A very simple gene regulatory circuit—a single gene regulating its own expression by the binding of its protein product to its own regulatory DNA. Simple schematic diagrams such as this are often used to summarize what we know (as in Figure 1–44), but they leave many questions unanswered. When the protein binds, does it inhibit or stimulate transcription? How steeply does the transcription rate depend on the protein concentration? How long, on average, does a molecule of the protein remain bound to the DNA? How long does it take to make each molecule of mRNA or protein, and how quickly does each type of molecule get degraded? Mathematical modeling shows that we need quantitative answers to all these and other questions before we can predict the behavior of even this single-gene system. For different parameter values, the system may settle to a unique steady state; or it may behave as a switch, capable of existing in one or other of a set of alternative states; or it may oscillate; or it may show large random fluctuations.



changes in parameters can cause radical changes in outcome. To go from a circuit diagram to a prediction of the behavior of the system, we need detailed quantitative information, and to draw deductions from that information we need mathematics and computers.

These tools for quantitative reasoning are essential, but they are not all-powerful. You might think that, knowing how each protein influences each other protein, and how the expression of each gene is regulated by the products of others, we should soon be able to calculate how the cell as a whole will behave, just as an astronomer can calculate the orbits of the planets, or a chemical engineer can calculate the flows through a chemical plant. But any attempt to perform this feat for an entire living cell rapidly reveals the limits of our present state of knowledge. The information we have, plentiful as it is, is full of gaps and uncertainties. Moreover, it is largely qualitative rather than quantitative. Most often, cell biologists studying the cell's control systems sum up their knowledge in simple schematic diagrams—this book is full of them—rather than in numbers, graphs, and differential equations. To progress from qualitative descriptions and intuitive reasoning to quantitative descriptions and mathematical deduction is one of the biggest challenges for contemporary cell biology. So far, the challenge has been met only for a few very simple fragments of the machinery of living cells—subsystems involving a handful of different proteins, or two or three cross-regulatory genes, where theory and experiment can go closely hand in hand. We shall discuss some of these examples later in the book.

Arabidopsis Has Been Chosen Out of 300,000 Species As a Model Plant

The large multicellular organisms that we see around us—the flowers and trees and animals—seem fantastically varied, but they are much closer to one another in their evolutionary origins, and more similar in their basic cell biology, than the great host of microscopic single-celled organisms. Thus, while bacteria and eucaryotes are separated by more than 3000 million years of divergent evolution, vertebrates and insects are separated by about 700 million years, fish and mammals by about 450 million years, and the different species of flowering plants by only about 150 million years.

Because of the close evolutionary relationship between all flowering plants, we can, once again, get insight into the cell and molecular biology of this whole class of organisms by focusing on just one or a few species for detailed analysis. Out of the several hundred thousand species of flowering plants on Earth today, molecular biologists have chosen to concentrate their efforts on a small weed, the common Thale cress *Arabidopsis thaliana* (**Figure 1–46**), which can be grown indoors in large numbers, and produces thousands of offspring per plant after 8–10 weeks. *Arabidopsis* has a genome of approximately 140 million nucleotide pairs, about 11 times as much as yeast, and its complete sequence is known.



Figure 1–46 ***Arabidopsis thaliana,*** **the plant chosen as the primary model for studying plant molecular genetics.** (Courtesy of Toni Hayden and the John Innes Foundation.)

The World of Animal Cells Is Represented By a Worm, a Fly, a Mouse, and a Human

Multicellular animals account for the majority of all named species of living organisms, and for the largest part of the biological research effort. Four species have emerged as the foremost model organisms for molecular genetic studies. In order of increasing size, they are the nematode worm *Caenorhabditis elegans*, the fly *Drosophila melanogaster*, the mouse *Mus musculus*, and the human, *Homo sapiens*. Each of these has had its genome sequenced.

Caenorhabditis elegans (**Figure 1–47**) is a small, harmless relative of the eelworm that attacks crops. With a life cycle of only a few days, an ability to survive in a freezer indefinitely in a state of suspended animation, a simple body plan, and an unusual life cycle that is well suited for genetic studies (described in Chapter 23), it is an ideal model organism. *C. elegans* develops with clockwork precision from a fertilized egg cell into an adult worm with exactly 959 body cells

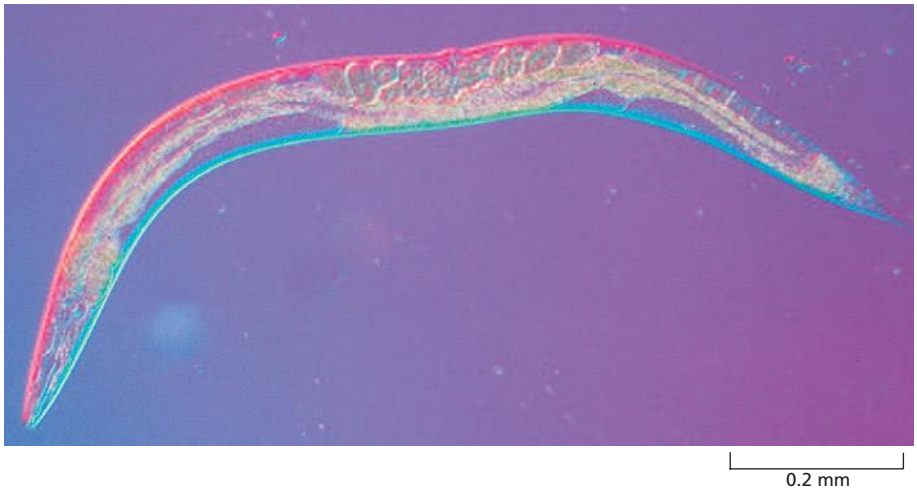


Figure 1–47 ***Caenorhabditis elegans,*** **the first multicellular organism to have its complete genome sequence determined.** This small nematode, about 1 mm long, lives in the soil. Most individuals are hermaphrodites, producing both eggs and sperm. The animal is viewed here using interference contrast optics, showing up the boundaries of the tissues in bright colors; the animal itself is not colored when viewed with ordinary lighting. (Courtesy of Ian Hope.)

(plus a variable number of egg and sperm cells)—an unusual degree of regularity for an animal. We now have a minutely detailed description of the sequence of events by which this occurs, as the cells divide, move, and change their characters according to strict and predictable rules. The genome of 97 million nucleotide pairs codes for about 19,000 proteins, and many mutants and other tools are available for the testing of gene functions. Although the worm has a body plan very different from our own, the conservation of biological mechanisms has been sufficient for the worm to be a model for many of the developmental and cell-biological processes that occur in the human body. Studies of the worm help us to understand, for example, the programs of cell division and cell death that determine the numbers of cells in the body—a topic of great importance in developmental biology and cancer research.

Studies in *Drosophila* Provide a Key to Vertebrate Development

The fruitfly *Drosophila melanogaster* (**Figure 1–48**) has been used as a model genetic organism for longer than any other; in fact, the foundations of classical genetics were built to a large extent on studies of this insect. Over 80 years ago, it provided, for example, definitive proof that genes—the abstract units of hereditary information—are carried on chromosomes, concrete physical objects whose behavior had been closely followed in the eucaryotic cell with the light microscope, but whose function was at first unknown. The proof depended on one of the many features that make *Drosophila* peculiarly convenient for genetics—the

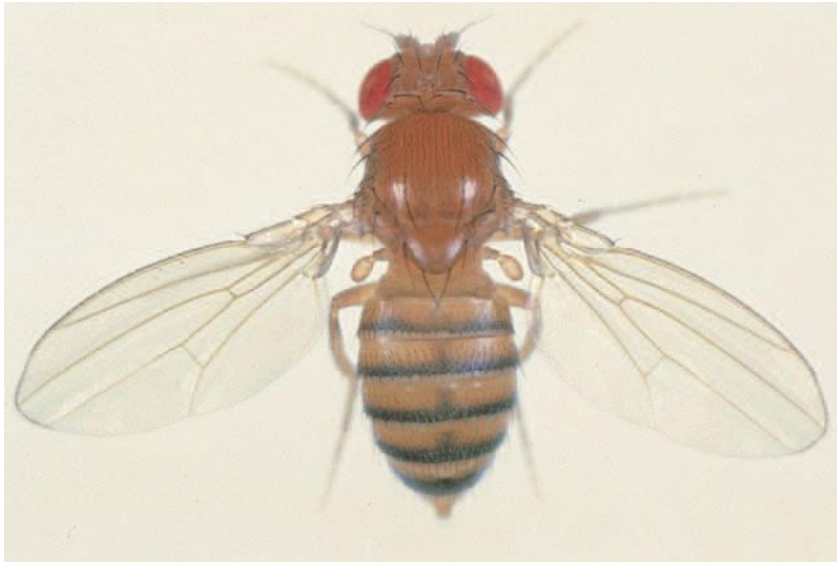


Figure 1–48 ***Drosophila melanogaster.*** Molecular genetic studies on this fly have provided the main key to understanding how all animals develop from a fertilized egg into an adult. (From E.B. Lewis, *Science* 221:cover, 1983. With permission from AAAS.)

giant chromosomes, with characteristic banded appearance, that are visible in some of its cells (**Figure 1–49**). Specific changes in the hereditary information, manifest in families of mutant flies, were found to correlate exactly with the loss or alteration of specific giant-chromosome bands.

In more recent times, *Drosophila*, more than any other organism, has shown us how to trace the chain of cause and effect from the genetic instructions encoded in the chromosomal DNA to the structure of the adult multicellular body. *Drosophila* mutants with body parts strangely misplaced or mispatterned provided the key to the identification and characterization of the genes required to make a properly structured body, with gut, limbs, eyes, and all the other parts in their correct places. Once these *Drosophila* genes were sequenced, the genomes of vertebrates could be scanned for homologs. These were found, and their functions in vertebrates were then tested by analyzing mice in which the genes had been mutated. The results, as we see later in the book, reveal an astonishing degree of similarity in the molecular mechanisms of insect and vertebrate development.

The majority of all named species of living organisms are insects. Even if *Drosophila* had nothing in common with vertebrates, but only with insects, it would still be an important model organism. But if understanding the molecular genetics of vertebrates is the goal, why not simply tackle the problem head-on? Why sidle up to it obliquely, through studies in *Drosophila*?

Drosophila requires only 9 days to progress from a fertilized egg to an adult; it is vastly easier and cheaper to breed than any vertebrate, and its genome is much smaller—about 170 million nucleotide pairs, compared with 3200 million for a human. This genome codes for about 14,000 proteins, and mutants can now be obtained for essentially any gene. But there is also another, deeper reason why genetic mechanisms that are hard to discover in a vertebrate are often readily revealed in the fly. This relates, as we now explain, to the frequency of gene duplication, which is substantially greater in vertebrate genomes than in the fly genome and has probably been crucial in making vertebrates the complex and subtle creatures that they are.



Figure 1–49 Giant chromosomes from salivary gland cells of *Drosophila*. Because many rounds of DNA replication have occurred without an intervening cell division, each of the chromosomes in these unusual cells contains over 1000 identical DNA molecules, all aligned in register. This makes them easy to see in the light microscope, where they display a characteristic and reproducible banding pattern. Specific bands can be identified as the locations of specific genes: a mutant fly with a region of the banding pattern missing shows a phenotype reflecting loss of the genes in that region. Genes that are being transcribed at a high rate correspond to bands with a "puffed" appearance. The bands stained dark brown in the micrograph are sites where a particular regulatory protein is bound to the DNA. (Courtesy of B. Zink and R. Paro, from R. Paro, *Trends Genet.* 6:416–421, 1990. With permission from Elsevier.)

The Vertebrate Genome Is a Product of Repeated Duplication

Almost every gene in the vertebrate genome has paralogs—other genes in the same genome that are unmistakably related and must have arisen by gene duplication. In many cases, a whole cluster of genes is closely related to similar clusters present elsewhere in the genome, suggesting that genes have been duplicated in linked groups rather than as isolated individuals. According to one hypothesis, at an early stage in the evolution of the vertebrates, the entire genome underwent duplication twice in succession, giving rise to four copies of every gene. In some groups of vertebrates, such as fish of the salmon and carp families (including the zebrafish, a popular research animal), it has been suggested that there was yet another duplication, creating an eightfold multiplicity of genes.

The precise course of vertebrate genome evolution remains uncertain, because many further evolutionary changes have occurred since these ancient events. Genes that were once identical have diverged; many of the gene copies have been lost through disruptive mutations; some have undergone further rounds of local duplication; and the genome, in each branch of the vertebrate family tree, has suffered repeated rearrangements, breaking up most of the original gene orderings. Comparison of the gene order in two related organisms, such as the human and the mouse, reveals that—on the time scale of vertebrate evolution—chromosomes frequently fuse and fragment to move large blocks of DNA sequence around. Indeed, it is possible, as we shall discuss in Chapter 7, that the present state of affairs is the result of many separate duplications of fragments of the genome, rather than duplications of the genome as a whole.

There is, however, no doubt that such whole-genome duplications do occur from time to time in evolution, for we can see recent instances in which duplicated chromosome sets are still clearly identifiable as such. The frog





Figure 1–50 Two species of the frog genus *Xenopus*. *X. tropicalis*, above, has an ordinary diploid genome; *X. laevis*, below, has twice as much DNA per cell. From the banding patterns of their chromosomes and the arrangement of genes along them, as well as from comparisons of gene sequences, it is clear that the large-genome species have evolved through duplications of the whole genome. These duplications are thought to have occurred in the aftermath of matings between frogs of slightly divergent *Xenopus* species. (Courtesy of E. Amaya, M. Offield and R. Grainger, *Trends Genet.* 14:253–255, 1998. With permission from Elsevier.)

genus *Xenopus*, for example, comprises a set of closely similar species related to one another by repeated duplications or triplications of the whole genome. Among these frogs are *X. tropicalis*, with an ordinary diploid genome; the common laboratory species *X. laevis*, with a duplicated genome and twice as much DNA per cell; and *X. ruwenzoriensis*, with a sixfold reduplication of the original genome and six times as much DNA per cell (108 chromosomes, compared with 36 in *X. laevis*, for example). These species are estimated to have diverged from one another within the past 120 million years (**Figure 1–50**).

Genetic Redundancy Is a Problem for Geneticists, But It Creates Opportunities for Evolving Organisms

Whatever the details of the evolutionary history, it is clear that most genes in the vertebrate genome exist in several versions that were once identical. The related genes often remain functionally interchangeable for many purposes. This phenomenon is called **genetic redundancy**. For the scientist struggling to discover all the genes involved in some particular process, it complicates the task. If gene A is mutated and no effect is seen, it cannot be concluded that gene A is functionally irrelevant—it may simply be that this gene normally works in parallel with its relatives, and these suffice for near-normal function even when gene A is defective. In the less repetitive genome of *Drosophila*, where gene duplication is less common, the analysis is more straightforward: single gene functions are revealed directly by the consequences of single-gene mutations (the single-engined plane stops flying when the engine fails).

Genome duplication has clearly allowed the development of more complex life forms; it provides an organism with a cornucopia of spare gene copies, which are free to mutate to serve divergent purposes. While one copy becomes optimized for use in the liver, say, another can become optimized for use in the brain or adapted for a novel purpose. In this way, the additional genes allow for increased complexity and sophistication. As the genes take on divergent functions, they cease to be redundant. Often, however, while the genes acquire individually specialized roles, they also continue to perform some aspects of their original core function in parallel, redundantly. Mutation of a single gene then causes a relatively minor abnormality that reveals only a part of the gene's function (**Figure 1–51**). Families of genes with divergent but partly overlapping functions are a pervasive feature of vertebrate molecular biology, and they are encountered repeatedly in this book.

The Mouse Serves as a Model for Mammals

Mammals have typically three or four times as many genes as *Drosophila,* a genome that is 20 times larger, and millions or billions of times as many cells in their adult bodies. In terms of genome size and function, cell biology, and molecular mechanisms, mammals are nevertheless a highly uniform group of organisms. Even anatomically, the differences among mammals are chiefly a matter of size and proportions; it is hard to think of a human body part that does not have a counterpart in elephants and mice, and vice versa. Evolution plays freely with quantitative features, but it does not readily change the logic of the structure.

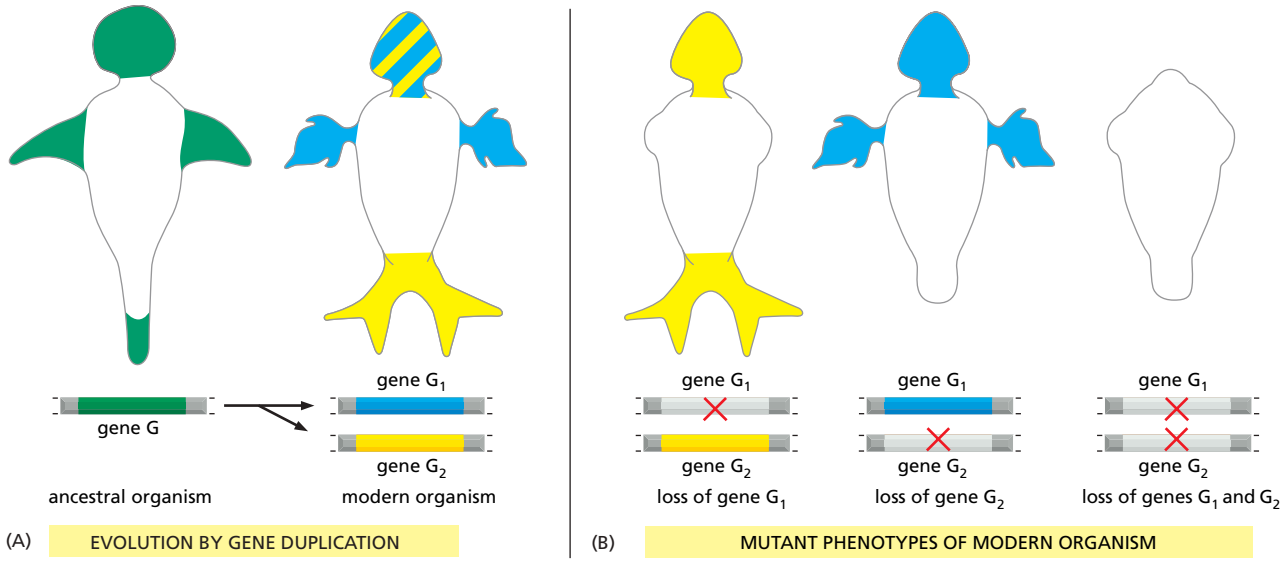


Figure 1–51 The consequences of gene duplication for mutational analyses of gene function. In this hypothetical example, an ancestral multicellular organism has a genome containing a single copy of gene G, which performs its function at several sites in the body, indicated in *green*. (A) Through gene duplication, a modern descendant of the ancestral organism has two copies of gene G, called G_1 and G_2. These have diverged somewhat in their patterns of expression and in their activities at the sites where they are expressed, but they still retain important similarities. At some sites, they are expressed together, and each independently performs the same old function as the ancestral gene G (alternating *green* and *yellow stripes);* at other sites, they are expressed alone and may serve new purposes. (B) Because of a functional overlap, the loss of one of the two genes by mutation *(red cross)* reveals only a part of its role; only the loss of both genes in the double mutant reveals the full range of processes for which these genes are responsible. Analogous principles apply to duplicated genes that operate in the same place (for example, in a single-celled organism) but are called into action together or individually in response to varying circumstances. Thus, gene duplications complicate genetic analyses in all organisms.

For a more exact measure of how closely mammalian species resemble one another genetically, we can compare the nucleotide sequences of corresponding (orthologous) genes, or the amino acid sequences of the proteins that these genes encode. The results for individual genes and proteins vary widely. But typically, if we line up the amino acid sequence of a human protein with that of the orthologous protein from, say, an elephant, about 85% of the amino acids are identical. A similar comparison between human and bird shows an amino acid identity of about 70%—twice as many differences, because the bird and the mammalian lineages have had twice as long to diverge as those of the elephant and the human (**Figure 1–52**).

The mouse, being small, hardy, and a rapid breeder, has become the foremost model organism for experimental studies of vertebrate molecular genetics. Many naturally occurring mutations are known, often mimicking the effects of corresponding mutations in humans (**Figure 1–53**). Methods have been developed, moreover, to test the function of any chosen mouse gene, or of any noncoding portion of the mouse genome, by artificially creating mutations in it, as we explain later in the book.

One made-to-order mutant mouse can provide a wealth of information for the cell biologist. It reveals the effects of the chosen mutation in a host of different contexts, simultaneously testing the action of the gene in all the different kinds of cells in the body that could in principle be affected.

Humans Report on Their Own Peculiarities

As humans, we have a special interest in the human genome. We want to know the full set of parts from which we are made, and to discover how they work. But even if you were a mouse, preoccupied with the molecular biology of mice, humans would be attractive as model genetic organisms, because of one special property: through medical examinations and self-reporting, we catalog our own genetic (and other) disorders. The human population is enormous, consisting today of some 6 billion individuals, and this self-documenting property means that a huge database of information exists on human mutations. The complete human genome sequence of more than 3 billion nucleotide pairs has now been determined, making it easier than ever before to identify at a molecular level the precise gene responsible for each human mutant characteristic.

By drawing together the insights from humans, mice, flies, worms, yeasts, plants, and bacteria—using gene sequence similarities to map out the correspondences between one model organism and another—we enrich our understanding of them all.

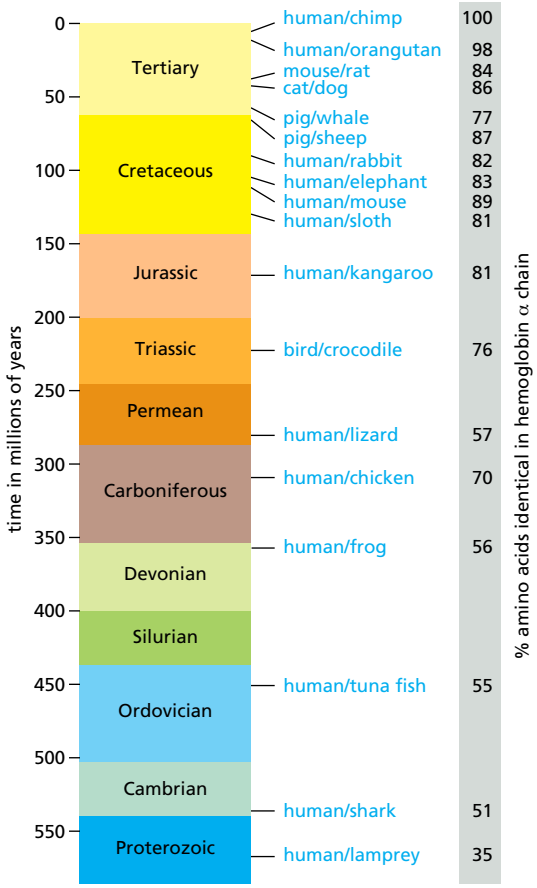


Figure 1–52 Times of divergence of different vertebrates. The scale on the left shows the estimated date and geological era of the last common ancestor of each specified pair of animals. Each time estimate is based on comparisons of the amino acid sequences of orthologous proteins; the longer a pair of animals have had to evolve independently, the smaller the percentage of amino acids that remain identical. Data from many different classes of proteins have been averaged to arrive at the final estimates, and the time scale has been calibrated to match the fossil evidence that the last common ancestor of mammals and birds lived 310 million years ago. The figures on the right give data on sequence divergence for one particular protein (chosen arbitrarily)—the α chain of hemoglobin. Note that although there is a clear general trend of increasing divergence with increasing time for this protein, there are also some irregularities. These reflect the randomness within the evolutionary process and, probably, the action of natural selection driving especially rapid changes of hemoglobin sequence in some organisms that experienced special physiological demands. On average, within any particular evolutionary lineage, hemoglobins accumulate changes at a rate of about 6 altered amino acids per 100 amino acids every 100 million years. Some proteins, subject to stricter functional constraints, evolve much more slowly than this, others as much as 5 times faster. All this gives rise to substantial uncertainties in estimates of divergence times, and some experts believe that the major groups of mammals diverged from one another as much as 60 million years more recently than shown here. (Adapted from S. Kumar and S.B. Hedges, *Nature* 392:917–920, 1998. With permission from Macmillan Publishers Ltd.)

We Are All Different in Detail

What precisely do we mean when we speak of *the* human genome? Whose genome? On average, any two people taken at random differ in about one or two in every 1000 nucleotide pairs in their DNA sequence. The Human Genome Project has arbitrarily selected DNA from a small number of anonymous individuals for sequencing. The human genome—the genome of the human species—is, properly speaking, a more complex thing, embracing the entire pool of variant genes that are found in the human population and continually exchanged and reassorted in the course of sexual reproduction. Ultimately, we can hope to document this variation too. Knowledge of it will help us understand, for example, why some people are prone to one disease, others to another; why some respond well to a drug, others badly. It will also provide new clues to our history—the population movements and minglings of our ancestors, the infections they suffered, the diets they ate. All these things leave traces in the variant forms of genes that have survived in human communities.





Figure 1–53 Human and mouse: similar genes and similar development. The human baby and the mouse shown here have similar white patches on their foreheads because both have mutations in the same gene (called *Kit)*, required for the development and maintenance of pigment cells. (Courtesy of R.A. Fleischman.)

Knowledge and understanding bring the power to intervene—with humans, to avoid or prevent disease; with plants, to create better crops; with bacteria, to turn them to our own uses. All these biological enterprises are linked, because the genetic information of all living organisms is written in the same language. The new-found ability of molecular biologists to read and decipher this language has already begun to transform our relationship to the living world. The account of cell biology in the subsequent chapters will, we hope, prepare you to understand, and possibly to contribute to, the great scientific adventure of the twenty-first century.

Summary

Eucaryotic cells, by definition, keep their DNA in a separate membrane-enclosed compartment, the nucleus. They have, in addition, a cytoskeleton for support and movement, elaborate intracellular compartments for digestion and secretion, the capacity (in many species) to engulf other cells, and a metabolism that depends on the oxidation of organic molecules by mitochondria. These properties suggest that eucaryotes may have originated as predators on other cells. Mitochondria—and, in plants, chloroplasts—contain their own genetic material, and evidently evolved from bacteria that were taken up into the cytoplasm of the eucaryotic cell and survived as symbionts. Eucaryotic cells have typically 3–30 times as many genes as procaryotes, and often thousands of times more noncoding DNA. The noncoding DNA allows for complex regulation of gene expression, as required for the construction of complex multicellular organisms. Many eucaryotes are, however, unicellular—among them the yeast Saccharomyces cerevisiae, *which serves as a simple model organism for eucaryotic cell biology, revealing the molecular basis of conserved fundamental processes such as the eucaryotic cell division cycle. A small number of other organisms have been chosen as primary models for multicellular plants and animals, and the sequencing of their entire genomes has opened the way to systematic and comprehensive analysis of gene functions, gene regulation, and genetic diversity. As a result of gene duplications during vertebrate evolution, vertebrate genomes contain multiple closely related homologs of most genes. This genetic redundancy has allowed diversification and specialization of genes for new purposes, but it also makes gene functions harder to decipher. There is less genetic redundancy in the nematode* Caenorhabditis elegans *and the fly* Drosophila melanogaster, *which have thus played a key part in revealing universal genetic mechanisms of animal development.*

PROBLEMS

Which statements are true? Explain why or why not.

1–1 The human hemoglobin genes, which are arranged in two clusters on two chromosomes, provide a good example of an orthologous set of genes.

1–2 Horizontal gene transfer is more prevalent in single-celled organisms than in multicellular organisms.

1–3 Most of the DNA sequences in a bacterial genome code for proteins, whereas most of the sequences in the human genome do not.

Discuss the following problems.

1–4 Since it was deciphered four decades ago, some have claimed that the genetic code must be a frozen accident, while others have argued that it was shaped by natural selection. A striking feature of the genetic code is its inherent resistance to the effects of mutation. For example, a change in the third position of a codon often specifies the same amino acid or one with similar chemical properties. The natural code resists mutation more effectively (is less susceptible to error) than most other possible versions, as illustrated in **Figure Q1–1**. Only one in a million computer-generated "random" codes is more error-resistant than the natural genetic code. Does the extraordinary mutation resistance of the genetic code argue in favor of its origin as a frozen accident or as a result of natural selection? Explain your reasoning.

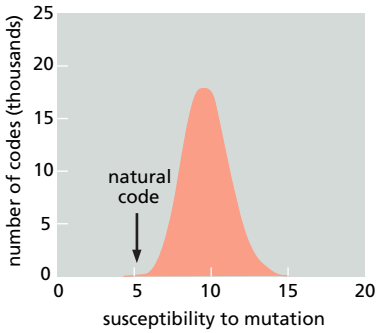


Figure Q1–1 Susceptibility of the natural code relative to millions of computer-generated codes (Problem 1–4). Susceptibility measures the average change in amino acid properties caused by random mutations. A small value indicates that mutations tend to cause minor changes. (Data courtesy of Steve Freeland.)

1–5 You have begun to characterize a sample obtained from the depths of the oceans on Europa, one of Jupiter's moons. Much to your surprise, the sample contains a life-form that grows well in a rich broth. Your preliminary analysis

shows that it is cellular and contains DNA, RNA, and protein. When you show your results to a colleague, she suggests that your sample was contaminated with an organism from Earth. What approaches might you try to distinguish between contamination and a novel cellular life-form based on DNA, RNA, and protein?

1–6 It is not so difficult to imagine what it means to feed on the organic molecules that living things produce. That is, after all, what we do. But what does it mean to "feed" on sunlight, as phototrophs do? Or, even stranger, to "feed" on rocks, as lithotrophs do? Where is the "food," for example, in the mixture of chemicals (H_2S, H_2, CO, Mn^+, Fe^{2+}, Ni^{2+}, CH_4, and NH_4^+) spewed forth from a hydrothermal vent?

1–7 How many possible different trees (branching patterns) can be drawn for eubacteria, archaea, and eucaryotes, assuming that they all arose from a common ancestor?

1–8 The genes for ribosomal RNA are highly conserved (relatively few sequence changes) in all organisms on Earth; thus, they have evolved very slowly over time. Were ribosomal RNA genes "born" perfect?

1–9 Genes participating in informational processes such as replication, transcription, and translation are transferred between species much less often than are genes involved in metabolism. The basis for this inequality is unclear at present, but one suggestion is that it relates to the underlying complexity. Informational processes tend to involve large aggregates of different gene products, whereas metabolic reactions are usually catalyzed by enzymes composed of a single protein. Why would the complexity of the underlying process—informational or metabolic—have any effect on the rate of horizontal gene transfer?

1–10 The process of gene transfer from the mitochondrial to the nuclear genome can be analyzed in plants. The respiratory gene *Cox2*, which encodes subunit 2 of cytochrome oxidase, was functionally transferred to the nucleus during flowering plant evolution. Extensive analyses of plant genera have pinpointed the time of appearance of the nuclear form of the gene and identified several likely intermediates in the ultimate loss from the mitochondrial genome. A summary of *Cox2* gene distributions between mitochondria and nuclei, along with data on their transcription, is shown in a phylogenetic context in **Figure Q1–2**.

A. Assuming that transfer of the mitochondrial gene to the nucleus occurred only once (an assumption supported by the structures of the nuclear genes), indicate the point in the phylogenetic tree where the transfer occurred.

B. Are there any examples of genera in which the transferred gene and the mitochondrial gene both appear functional? Indicate them.

C. What is the minimal number of times that the mitochondrial gene has been inactivated or lost? Indicate those events on the phylogenetic tree.

D. What is the minimal number of times that the nuclear gene has been inactivated or lost? Indicate those events on the phylogenetic tree.

E. Based on this information, propose a general scheme for transfer of mitochondrial genes to the nuclear genome.

1–11 When plant hemoglobin genes were first discovered in legumes, it was so surprising to find a gene typical of animal blood that it was hypothesized that the plant gene arose

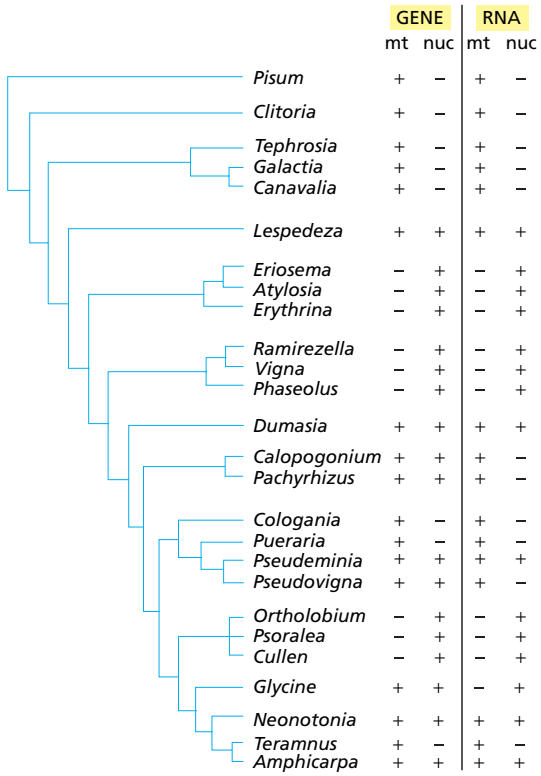


Figure Q1–2 Summary of *Cox2* gene distribution and transcript data in a phylogenetic context (Problem 1–10). The presence of the intact gene or a functional transcript is indicated by (+); the absence of the intact gene or a functional transcript is indicated by (–). mt, mitochondria; nuc, nuclei.

by horizontal transfer from an animal. Many more hemoglobin genes have now been sequenced, and a phylogenetic tree based on some of these sequences is shown in **Figure Q1–3**.

A. Does this tree support or refute the hypothesis that the plant hemoglobins arose by horizontal gene transfer?

B. Supposing that the plant hemoglobin genes were originally derived from a parasitic nematode, for example, what would you expect the phylogenetic tree to look like?

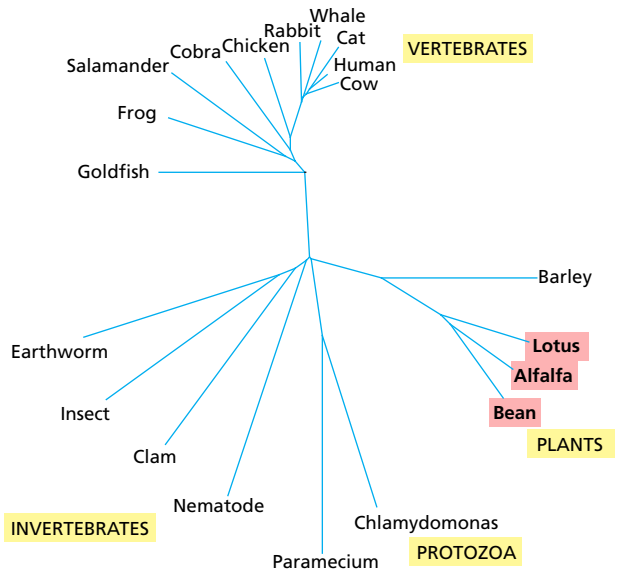


Figure Q1–3 Phylogenetic tree for hemoglobin genes from a variety of species (Problem 1–11). The legumes are highlighted in red.

1–12 Rates of evolution appear to vary in different lineages. For example, the rate of evolution in the rat lineage is significantly higher than in the human lineage. These rate differences are apparent whether one looks at changes in protein sequences that are subject to selective pressure or at changes in noncoding nucleotide sequences, which are not under obvious selection pressure. Can you offer one or more possible explanations for the slower rate of evolutionary change in the human lineage versus the rat lineage?

REFERENCES

General

Alberts B, Bray D, Hopkin K et al (2004) Essential Cell Biology, 2nd ed. New York: Garland Science.

Barton NH, Briggs DEG, Eisen JA et al (2007) Evolution. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Darwin C (1859) On the Origin of Species. London: Murray.

Graur D & Li W-H (1999) Fundamentals of Molecular Evolution, 2nd ed. Sunderland, MA: Sinauer Associates.

Madigan MT & Martinko JM (2005) Brock's Biology of Microorganisms, 11th ed. Englewood Cliffs, NJ: Prentice Hall.

Margulis L & Schwartz KV (1998) Five Kingdoms: An Illustrated Guide to the Phyla of Life on Earth, 3rd ed. New York: Freeman.

Watson JD, Baker TA, Bell SP et al (2007) Molecular Biology of the Gene, 6th ed. Menlo Park, CA: Benjamin-Cummings.

The Universal Features of Cells on Earth

Andersson SGE (2006) The bacterial world gets smaller. *Science* 314:259–260.

Brenner S, Jacob F & Meselson M (1961) An unstable intermediate carrying information from genes to ribosomes for protein synthesis. *Nature* 190:576–581.

Fraser CM, Gocayne JD, White O et al (1995) The minimal gene complement of *Mycoplasma genitalium*. *Science* 270:397–403.

Harris JK, Kelley ST, Spiegelman et al (2003) The genetic core of the universal ancestor. *Genome Res* 13:407–413.

Koonin EV (2005) Orthologs, paralogs, and evolutionary genomics. *Annu Rev Genet* 39:309–338.

Watson JD & Crick FHC (1953) Molecular structure of nucleic acids. A structure for deoxyribose nucleic acid. *Nature* 171:737–738.

Yusupov MM,Yusupova GZ, Baucom A et al (2001) Crystal structure of the ribosome at 5.5 Å resolution. *Science* 292:883–896.

The Diversity of Genomes and the Tree of Life

Blattner FR, Plunkett G, Bloch CA et al (1997) The complete genome sequence of *Escherichia coli* K-12. *Science* 277:1453–1474.

Boucher Y, Douady CJ, Papke RT et al (2003) Lateral gene transfer and the origins of prokaryotic groups. *Annu Rev Genet* 37:283–328.

Cole ST, Brosch R, Parkhill J et al (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 393:537–544.

Dixon B (1994) Power Unseen: How Microbes Rule the World. Oxford: Freeman.

Kerr RA (1997) Life goes to extremes in the deep earth—and elsewhere? *Science* 276:703–704.

Lee TI, Rinaldi NJ, Robert F et al (2002) Transcriptional regulatory networks in *Saccharomyces cerevisiae*. *Science* 298:799–804.

Olsen GJ & Woese CR (1997) Archaeal genomics: an overview. *Cell* 89:991–994.

Pace NR (1997) A molecular view of microbial diversity and the biosphere. *Science* 276:734–740.

Woese C (1998) The universal ancestor. *Proc Natl Acad Sci USA* 95:6854–6859.

Genetic Information in Eucaryotes

Adams MD, Celniker SE, Holt RA et al (2000) The genome sequence of *Drosophila melanogaster*. *Science* 287:2185–2195.

Andersson SG, Zomorodipour A, Andersson JO et al (1998) The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature* 396:133–140.

The *Arabidopsis* Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408:796–815.

Carroll SB, Grenier JK & Weatherbee SD (2005) From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design, 2nd ed. Maldon, MA: Blackwell Science.

de Duve C (2007) The origin of eukaryotes: a reappraisal. *Nature Rev Genet* 8:395-403.

Delsuc F, Brinkmann H & Philippe H (2005) Phylogenomics and the reconstruction of the tree of life. *Nature Rev Genet* 6:361–375.

DeRisi JL, Iyer VR & Brown PO (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278:680–686.

Gabriel SB, Schaffner SF, Nguyen H et al (2002) The structure of haplotype blocks in the human genome. *Science* 296:2225–2229.

Goffeau A, Barrell BG, Bussey H et al (1996) Life with 6000 genes. *Science* 274:546–567.

International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. *Nature* 409:860–921.

Kellis M, Birren BW & Lander ES (2004) Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*. *Nature* 428:617–624.

Lynch M & Conery JS (2000) The evolutionary fate and consequences of duplicate genes. *Science* 290:1151–1155.

Mulley J & Holland P (2004) Comparative genomics: Small genome, big insights. *Nature* 431:916–917.

National Center for Biotechnology Information. http://www.ncbi.nlm.nih.gov/

Owens K & King MC (1999) Genomic views of human history. *Science* 286:451–453.

Palmer JD & Delwiche CF (1996) Second-hand chloroplasts and the case of the disappearing nucleus. *Proc Natl Acad Sci USA* 93:7432–7435.

Pennisi E (2004) The birth of the nucleus. *Science* 305:766–768.

Plasterk RH (1999) The year of the worm. *BioEssays* 21:105–109.

Reed FA & Tishkoff SA (2006) African human diversity, origins and migrations. *Curr Opin Genet Dev* 16:597–605.

Rubin GM, Yandell MD, Wortman JR et al (2000) Comparative genomics of the eukaryotes. *Science* 287:2204–2215.

Stillman B & Stewart D (2003) The genome of *Homo sapiens*. (Cold Spring Harbor Symp. Quant. Biol. LXVIII). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

The *C. elegans* Sequencing Consortium (1998) Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 282:2012–2018.

Tinsley RC & Kobel HR (eds) (1996) The Biology of *Xenopus*. Oxford: Clarendon Press.

Tyson JJ, Chen KC & Novak B (2003) Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. *Curr Opin Cell Biol* 15:221–231.

Venter JC, Adams MD, Myers EW et al (2001) The sequence of the human genome. *Science* 291:1304–1351.

Cell Chemistry and Biosynthesis

2

It is at first sight difficult to accept the idea that each of the living creatures described in Chapter 1 is merely a chemical system. The incredible diversity of living forms, their seemingly purposeful behavior, and their ability to grow and reproduce appear to set them apart from the world of solids, liquids, and gases that chemistry normally describes. Indeed, until the nineteenth century animals were believed to contain a Vital Force—an "animus"—that was responsible for their distinctive properties.

We now know there is nothing in living organisms that disobeys chemical and physical laws. However, the chemistry of life is special. First, it is based overwhelmingly on carbon compounds, whose study is therefore known as *organic chemistry*. Second, cells are 70 percent water, and life depends largely on chemical reactions that take place in aqueous solution. Third, and most important, cell chemistry is enormously complex: even the simplest cell is vastly more complicated in its chemistry than any other chemical system known. Although cells contain a variety of small carbon-containing molecules, most of the carbon atoms in cells are incorporated into enormous *polymeric molecules*—chains of chemical subunits linked end-to-end. It is the unique properties of these macromolecules that enable cells and organisms to grow and reproduce—as well as to do all the other things that are characteristic of life.

In This Chapter

THE CHEMICAL COMPONENTS OF A CELL 45

CATALYSIS AND THE USE OF ENERGY BY CELLS 65

HOW CELLS OBTAIN ENERGY FROM FOOD 88

THE CHEMICAL COMPONENTS OF A CELL

Matter is made of combinations of *elements*—substances such as hydrogen or carbon that cannot be broken down or converted into other substances by chemical means. The smallest particle of an element that still retains its distinctive chemical properties is an *atom* (**Figure 2–1**). However, the characteristics of substances other than pure elements—including the materials from which living cells are made—depend on the way their atoms are linked together in groups to form *molecules*. In order to understand how living organisms are built from inanimate matter, therefore, it is crucial to know how all of the chemical bonds that hold atoms together in molecules are formed.

Cells Are Made From a Few Types of Atoms

The **atomic weight** of an atom, or the **molecular weight** of a molecule, is its mass relative to that of a hydrogen atom. This is essentially equal to the number of protons plus neutrons that the atom or molecule contains, since the electrons are much lighter and contribute almost nothing to the total. Thus the major isotope of carbon has an atomic weight of 12 and is symbolized as ^{12}C, whereas an unstable isotope of carbon has an atomic weight of 14 and is written as ^{14}C. The mass of an atom or a molecule is often specified in *daltons*, one dalton being an atomic mass unit approximately equal to the mass of a hydrogen atom.

Atoms are so small that it is hard to imagine their size. An individual carbon atom is roughly 0.2 nm in diameter, so that it would take about 5 million of them, laid out in a straight line, to span a millimeter. One proton or neutron weighs

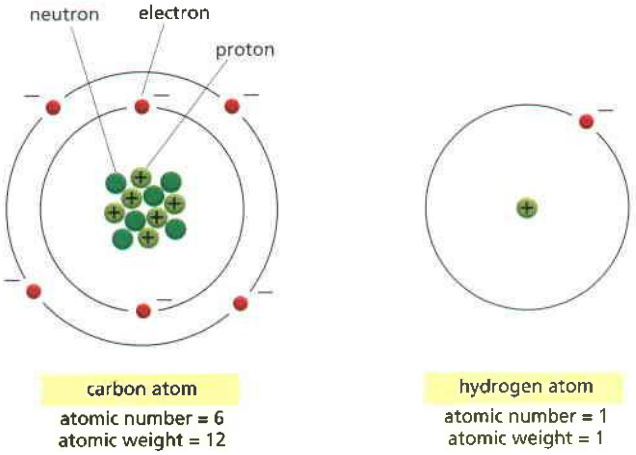


Figure 2–1 Highly schematic representations of an atom of carbon and an atom of hydrogen. The *nucleus* of every atom except hydrogen consists of both positively charged *protons* and electrically neutral *neutrons*. The number of electrons in an atom is equal to its number of protons (the *atomic number*), so that the atom has no net charge. Because it is the electrons that determine the chemical behavior of an atom, all of the atoms of a given element have the same atomic number.

Neutrons are uncharged subatomic particles of essentially the same mass as protons. They contribute to the structural stability of the nucleus—if there are too many or too few, the nucleus may disintegrate by radioactive decay—but they do not alter the chemical properties of the atom. Because of neutrons, an element can exist in several physically distinguishable but chemically identical forms, called *isotopes*, each isotope having a different number of neutrons but the same number of protons. Multiple isotopes of almost all the elements occur naturally, including some that are unstable. For example, while most carbon on Earth exists as the stable isotope carbon 12, with six protons and six neutrons, there are also small amounts of an unstable isotope, the radioactive carbon 14, whose atoms have six protons and eight neutrons. Carbon 14 undergoes radioactive decay at a slow but steady rate. This forms the basis for a technique known as carbon 14 dating, which is used in archaeology to determine the time of origin of organic materials.

The neutrons, protons, and electrons are in reality minute in relation to the atom as a whole; their size is greatly exaggerated here. In addition, the diameter of the nucleus is only about 10^{-4} that of the electron cloud. Finally, although the electrons are shown here as individual particles, in reality their behavior is governed by the laws of quantum mechanics, and there is no way of predicting exactly where an electron is at any given instant of time.

approximately $1/(6 \times 10^{23})$ gram, so one gram of hydrogen contains 6×10^{23} atoms. This huge number (6×10^{23}, called **Avogadro's number**) is the key scale factor describing the relationship between everyday quantities and quantities measured in terms of individual atoms or molecules. If a substance has a molecular weight of X, 6×10^{23} molecules of it will have a mass of X grams. This quantity is called one **mole** of the substance (Figure 2–2).

There are 89 naturally occurring elements, each differing from the others in the number of protons and electrons in its atoms. Living organisms, however, are made of only a small selection of these elements, four of which—carbon (C), hydrogen (H), nitrogen (N), and oxygen (O)—make up 96.5% of an organism's weight. This composition differs markedly from that of the nonliving inorganic environment (Figure 2–3) and is evidence of a distinctive type of chemistry.

The Outermost Electrons Determine How Atoms Interact

To understand how atoms bond together to form the molecules that make up living organisms, we focus on their electrons. Protons and neutrons are welded tightly to one another in the nucleus and change partners only under extreme conditions—during radioactive decay, for example, or in the interior of the sun or of a nuclear reactor. In living tissues, it is only the electrons of an atom that undergo rearrangements. They form the exterior of an atom and specify the rules of chemistry by which atoms combine to form molecules.

Electrons are in continuous motion around the nucleus, but motions on this submicroscopic scale obey very different laws from those familiar in everyday life. These laws dictate that electrons in an atom can exist only in certain discrete states, called orbitals, and that there is a strict limit to the number of electrons that can be accommodated in an orbital of a given type—a so-called *electron shell*. The electrons closest on average to the positive nucleus are attracted most strongly to it and occupy the innermost, most tightly bound shell. This shell holds a maximum of two electrons. The second shell is farther away from the nucleus, and its electrons are less tightly bound. This second shell holds up to eight electrons. The third shell contains electrons that are even less tightly bound; it also holds up to eight electrons. The fourth and fifth shells can hold 18 electrons each. Atoms with more than four shells are very rare in biological molecules.

The electron arrangement of an atom is most stable when all the electrons are in the most tightly bound states that are possible—that is, when they occupy the innermost shells. Therefore, with certain exceptions in the larger atoms, the electrons of an atom fill the orbitals in order—the first shell before the second, the second before the third, and so on. An atom whose outermost shell is entirely filled with electrons is especially stable and therefore chemically unreactive. Examples are helium with 2 electrons, neon with 2 + 8, and argon with 2 + 8 + 8; these are all inert gases. Hydrogen, by contrast, with only one electron

and only a half-filled shell, is highly reactive. Likewise, the other atoms found in living tissues have incomplete outer electron shells and can donate, accept, or share electrons with each other to form both molecules and ions (**Figure 2–4**).

Because an unfilled electron shell is less stable than a filled one, atoms with incomplete outer shells tend to interact with other atoms in a way that causes them to either gain or lose enough electrons to achieve a completed outermost shell. This electron exchange occurs either by transferring electrons from one atom to another or by sharing electrons between two atoms. These two strategies generate two types of **chemical bonds** between atoms: an *ionic bond* is formed when electrons are donated by one atom to another, whereas a *covalent bond* is formed when two atoms share a pair of electrons (**Figure 2–5**). Often, the pair of electrons is shared unequally, with a partial transfer between two atoms that attract electrons differently—one more *electronegative* than the other: this intermediate strategy results in a *polar covalent bond*, as we shall discuss later.

An H atom, which needs only one electron to fill its shell, generally acquires it by electron sharing, forming one covalent bond with another atom; often this bond is polar—meaning that the electrons are shared unequally. The other common elements in living cells—C, N, and O, with an incomplete second shell, and P and S, with an incomplete third shell (see Figure 2–4)—generally share electrons and achieve a filled outer shell of eight electrons by forming several covalent bonds. The number of electrons that an atom must acquire or lose (either by sharing or by transfer) to fill its outer shell is known as its *valence*.

The crucial role of the outer electron shell in determining the chemical properties of an element means that, when the elements are listed in order of their atomic number, there is a periodic recurrence of elements with similar properties: an element with, say, an incomplete second shell containing one electron will behave in much the same way as an element that has filled its second shell

A **mole** is X grams of a substance, where X is its relative molecular mass (molecular weight). A mole will contain 6×10^{23} molecules of the substance.

1 mole of carbon weighs 12 g
1 mole of glucose weighs 180 g
1 mole of sodium chloride weighs 58 g

Molar solutions have a concentration of 1 mole of the substance in 1 liter of solution. A molar solution (denoted as 1 M) of glucose, for example, has 180 g/l, while a millimolar solution (1 mM) has 180 mg/l.

The standard abbreviation for gram is g; the abbreviation for liter is l.

Figure 2–2 Moles and molar solutions.

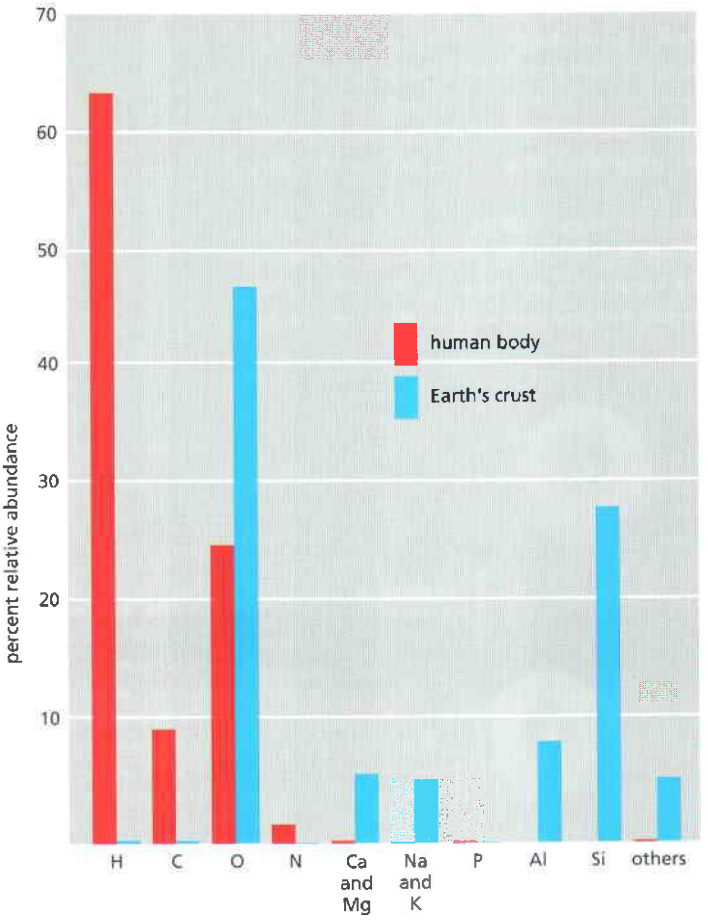


Figure 2–3 The abundances of some chemical elements in the nonliving world (the Earth's crust) compared with their abundances in the tissues of an animal. The abundance of each element is expressed as a percentage of the total number of atoms present including water. Thus, because of the abundance of water, more than 60% of the atoms in a living organism are hydrogen atoms. The relative abundance of elements is similar in all living things.

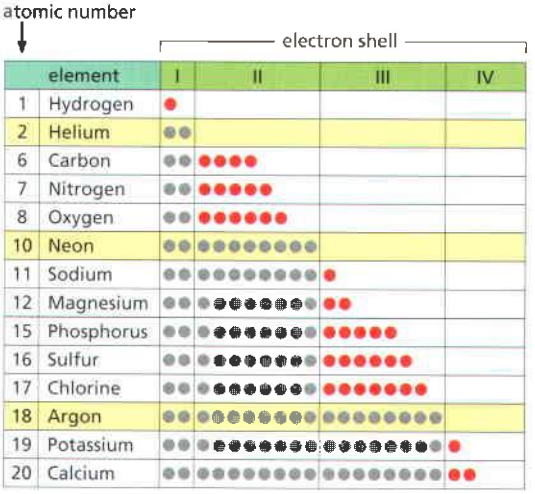


Figure 2–4 Filled and unfilled electron shells in some common elements. All the elements commonly found in living organisms have unfilled outermost shells *(red)* and can thus participate in chemical reactions with other atoms. For comparison, some elements that have only filled shells *(yellow)* are shown; these are chemically unreactive.

and has an incomplete third shell containing one electron. The metals, for example, have incomplete outer shells with just one or a few electrons, whereas, as we have just seen, the inert gases have full outer shells. This pattern gives rise to the famous *periodic table* of the elements, presented in Figure 2–6 with the elements found in living organisms highlighted.

Covalent Bonds Form by the Sharing of Electrons

All the characteristics of a cell depend on the molecules it contains. A **molecule** is defined as a cluster of atoms held together by **covalent bonds**; here electrons are shared between atoms to complete the outer shells, rather than being transferred between them. In the simplest possible molecule—a molecule of hydrogen (H_2)—two H atoms, each with a single electron, share two electrons, which is the number required to fill the first shell. These shared electrons form a cloud of negative charge that is densest between the two positively charged nuclei and helps to hold them together, in opposition to the mutual repulsion between like charges that would otherwise force them apart. The attractive and repulsive forces are in balance when the nuclei are separated by a characteristic distance, called the *bond length*.

Another property of any bond—covalent or noncovalent—is its *bond strength*, which is measured by the amount of energy that must be supplied to break that bond. This is often expressed in units of kilocalories per mole (kcal/mole), where a kilocalorie is the amount of energy needed to raise the temperature of one liter

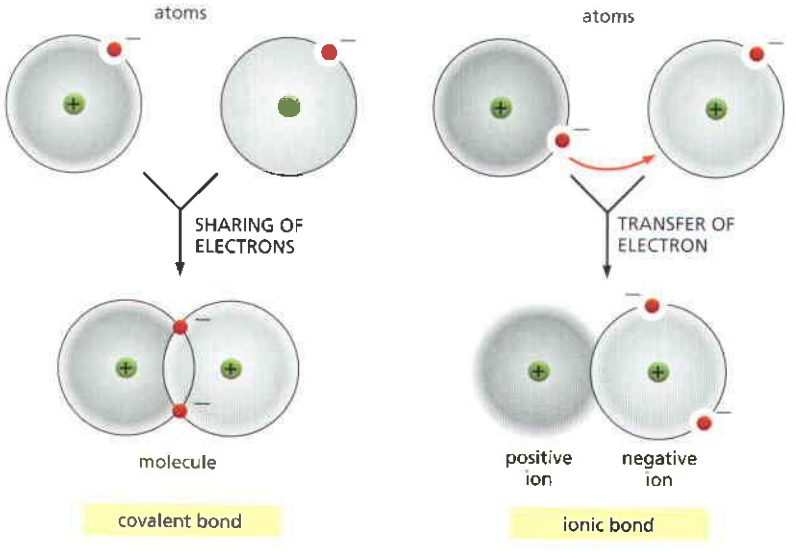


Figure 2–5 Comparison of covalent and ionic bonds. Atoms can attain a more stable arrangement of electrons in their outermost shell by interacting with one another. An ionic bond is formed when electrons are transferred from one atom to the other. A covalent bond is formed when electrons are shared between atoms. The two cases shown represent extremes; often, covalent bonds form with a partial transfer (unequal sharing of electrons), resulting in a polar covalent bond (see Figure 2–43).

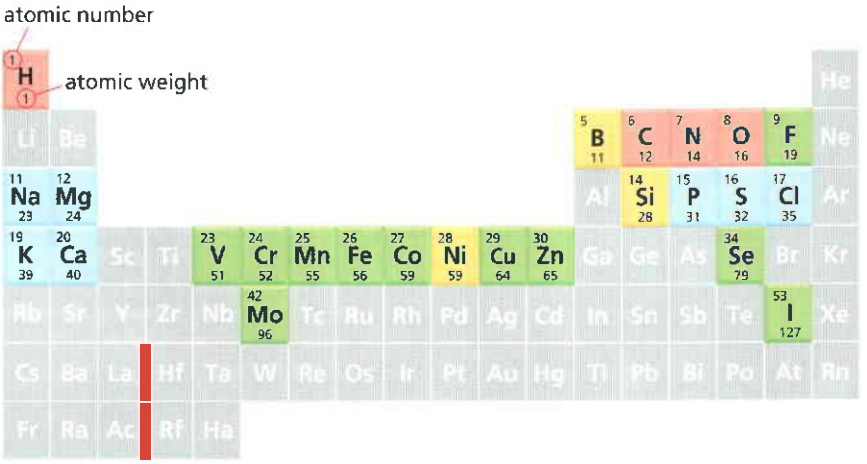


Figure 2–6 Elements ordered by their atomic number form the periodic table. Elements fall into groups that show similar properties based on the number of electrons each element possesses in its outer shell. For example, Mg and Ca tend to give away the two electrons in their outer shells; C, N, and O complete their second shells by sharing electrons. The four elements highlighted in *red* constitute 99% of the total number of atoms present in the human body. An additional seven elements, highlighted in *blue*, together represent about 0.9% of the total. Other elements, shown in *green*, are required in trace amounts by humans. It remains unclear whether those elements shown in *yellow* are essential in humans or not. The chemistry of life, it seems, is therefore predominantly the chemistry of lighter elements.

Atomic weights, given by the sum of the protons and neutrons in the atomic nucleus, will vary with the particular isotope of the element. The atomic weights shown here are those of the most common isotope of each element.

of water by one degree Celsius (centigrade). Thus if 1 kilocalorie must be supplied to break 6×10^{23} bonds of a specific type (that is, 1 mole of these bonds), then the strength of that bond is 1 kcal/mole. An equivalent, widely used measure of energy is the kilojoule, which is equal to 0.239 kilocalories.

To understand bond strengths, it is helpful to compare them with the average energies of the impacts that molecules are constantly experiencing from collisions with other molecules in their environment (their thermal, or heat, energy), as well as with other sources of biological energy such as light and glucose oxidation (Figure 2–7). Typical covalent bonds are stronger than the thermal energies by a factor of 100, so they resist being pulled apart by thermal motions and are normally broken only during specific chemical reactions with other atoms and molecules. The making and breaking of covalent bonds are violent events, and in living cells they are carefully controlled by highly specific catalysts, called *enzymes*. Noncovalent bonds as a rule are much weaker; we shall see later that they are important in the cell in the many situations where molecules have to associate and dissociate readily to carry out their functions.

Whereas an H atom can form only a single covalent bond, the other common atoms that form covalent bonds in cells—O, N, S, and P, as well as the all-important C atom—can form more than one. The outermost shell of these atoms, as we have seen, can accommodate up to eight electrons, and they form covalent bonds with as many other atoms as necessary to reach this number. Oxygen, with six electrons in its outer shell, is most stable when it acquires an extra two electrons by sharing with other atoms and therefore forms up to two covalent bonds. Nitrogen, with five outer electrons, forms a maximum of three covalent bonds, while carbon, with four outer electrons, forms up to four covalent bonds—thus sharing four pairs of electrons (see Figure 2–4).

When one atom forms covalent bonds with several others, these multiple bonds have definite arrangements in space relative to one another, reflecting the orientations of the orbits of the shared electrons. The covalent bonds of such an atom are therefore characterized by specific bond angles as well as by bond lengths and bond energies (Figure 2–8). The four covalent bonds that can form around a carbon atom, for example, are arranged as if pointing to the four corners of a regular tetrahedron. The precise orientation of covalent bonds forms the basis for the three-dimensional geometry of organic molecules.

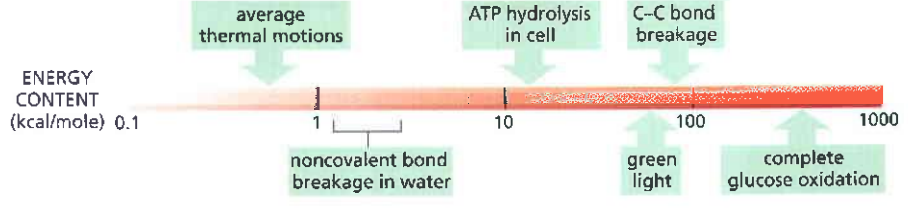


Figure 2–7 Some energies important for cells. Note that these energies are compared on a logarithmic scale.

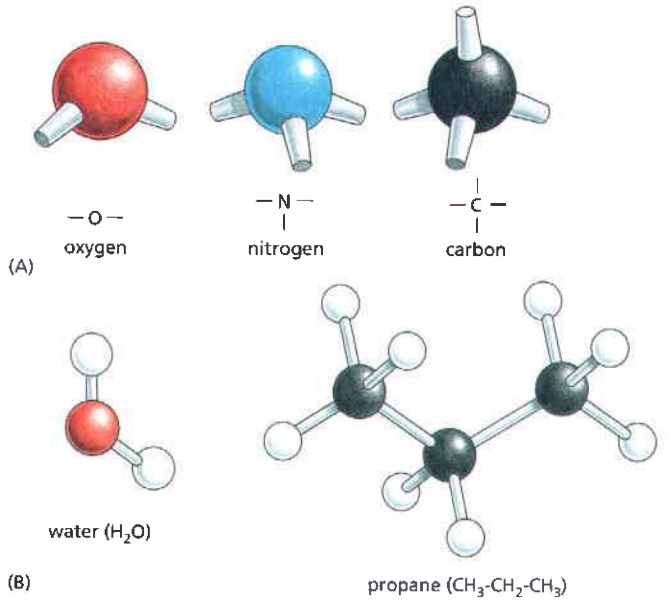


Figure 2–8 The geometry of covalent bonds. (A) The spatial arrangement of the covalent bonds that can be formed by oxygen, nitrogen, and carbon. (B) Molecules formed from these atoms have a precise three-dimensional structure, as shown here by ball-and-stick models for water and propane. A structure can be specified by the bond angles and bond lengths for each covalent linkage. The atoms are colored according to the following, generally used convention: H, *white*; C, *black*; O, *red*; N, *blue*.

There Are Different Types of Covalent Bonds

Most covalent bonds involve the sharing of two electrons, one donated by each participating atom; these are called *single bonds*. Some covalent bonds, however, involve the sharing of more than one pair of electrons. Four electrons can be shared, for example, two coming from each participating atom; such a bond is called a *double bond*. Double bonds are shorter and stronger than single bonds and have a characteristic effect on the three-dimensional geometry of molecules containing them. A single covalent bond between two atoms generally allows the rotation of one part of a molecule relative to the other around the bond axis. A double bond prevents such rotation, producing a more rigid and less flexible arrangement of atoms (Figure 2–9 and Panel 2–1, pp. 106–107).

In some molecules, electrons are shared among three or more atoms, producing bonds that have a hybrid character intermediate between single and double bonds. The highly stable benzene molecule, for example, consists of a ring of six carbon atoms in which the bonding electrons are evenly distributed (although usually depicted as an alternating sequence of single and double bonds, as shown in Panel 2–1).

When the atoms joined by a single covalent bond belong to different elements, the two atoms usually attract the shared electrons to different degrees. Compared with a C atom, for example, O and N atoms attract electrons relatively strongly, whereas an H atom attracts electrons more weakly. By definition, a **polar** structure (in the electrical sense) is one with positive charge concentrated toward one end (the positive pole) and negative charge concentrated toward the other (the negative pole). Covalent bonds in which the electrons are shared unequally in this way are therefore known as *polar covalent bonds* (Figure 2–10). For example, the covalent bond between oxygen and hydrogen, –O–H, or between nitrogen and hydrogen, –N–H, is polar, whereas that between carbon and hydrogen, –C–H, has the electrons attracted much more equally by both atoms and is relatively nonpolar.

Polar covalent bonds are extremely important in biology because they create *permanent dipoles* that allow molecules to interact through electrical forces. Any large molecule with many polar groups will have a pattern of partial positive and negative charges on its surface. When such a molecule encounters a second molecule with a complementary set of charges, the two molecules will be attracted to each other by electrostatic interactions that resemble (but are weaker than) the ionic bonds discussed previously.

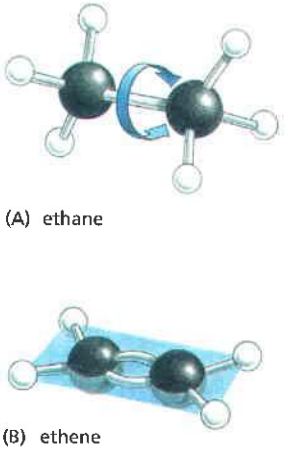


Figure 2–9 Carbon–carbon double bonds and single bonds compared. (A) The ethane molecule, with a single covalent bond between the two carbon atoms, illustrates the tetrahedral arrangement of single covalent bonds formed by carbon. One of the CH_3 groups joined by the covalent bond can rotate relative to the other around the bond axis. (B) The double bond between the two carbon atoms in a molecule of ethene (ethylene) alters the bond geometry of the carbon atoms and brings all the atoms into the same plane *(blue)*; the double bond prevents the rotation of one CH_2 group relative to the other.

An Atom Often Behaves as if It Has a Fixed Radius

When a covalent bond forms between two atoms, the sharing of electrons brings the nuclei of these atoms unusually close together. But most of the atoms that are rapidly jostling each other in cells are located in separate molecules. What happens when two such atoms touch? RoshanKetab 021-66950639

For simplicity and clarity, atoms and molecules are usually represented schematically—either as a line drawing of the structural formula or as a ball-and-stick model. *Space-filling models*, however, give us a more accurate representation of molecular structure. In these models, a solid envelope represents the radius of the electron cloud at which strong repulsive forces prevent a closer approach of any second, non-bonded atom—the so-called *van der Waals radius* for an atom. This is possible because the amount of repulsion increases very steeply as two such atoms approach each other closely. At slightly greater distances, any two atoms will experience a weak attractive force, known as a *van der Waals attraction*. As a result, there is a distance at which repulsive and attractive forces precisely balance to produce an energy minimum in each atom's interaction with an atom of a second, non-bonded element (**Figure 2–11**).

Depending on the intended purpose, we shall represent small molecules as line drawings, ball-and-stick models, or space-filling models. For comparison, the water molecule is represented in all three ways in **Figure 2–12**. When representing very large molecules, such as proteins, we shall often need to further simplify the model used (see, for example, Panel 3–2, pp. 132–133).

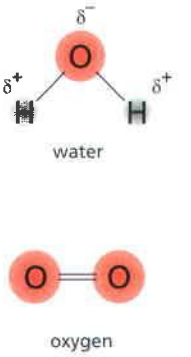


Figure 2–10 Polar and nonpolar covalent bonds. The electron distributions in the polar water molecule (H_2O) and the nonpolar oxygen molecule (O_2) are compared (δ^+, partial positive charge; δ^-, partial negative charge).

Water Is the Most Abundant Substance in Cells

Water accounts for about 70% of a cell's weight, and most intracellular reactions occur in an aqueous environment. Life on Earth began in the ocean, and the conditions in that primeval environment put a permanent stamp on the chemistry of living things. Life therefore hinges on the properties of water.

In each water molecule (H_2O) the two H atoms are linked to the O atom by covalent bonds (see Figure 2–12). The two bonds are highly polar because the O is strongly attractive for electrons, whereas the H is only weakly attractive. Consequently, there is an unequal distribution of electrons in a water molecule, with a preponderance of positive charge on the two H atoms and of negative charge on the O (see Figure 2–10). When a positively charged region of one water molecule (that is, one of its H atoms) approaches a negatively charged region (that is, the O) of a second water molecule, the electrical attraction between them can result in a weak bond called a *hydrogen bond* (see Figure 2–15). These bonds are much weaker than covalent bonds and are easily broken by the random thermal motions due to the heat energy of the molecules, so each bond lasts only a short time. But the combined effect of many weak bonds can be profound. Each water molecule can form hydrogen bonds through its two H atoms to two other water molecules, producing a network in which hydrogen bonds are being continually broken and formed (Panel 2–2, pp. 108–109). It is only because of the

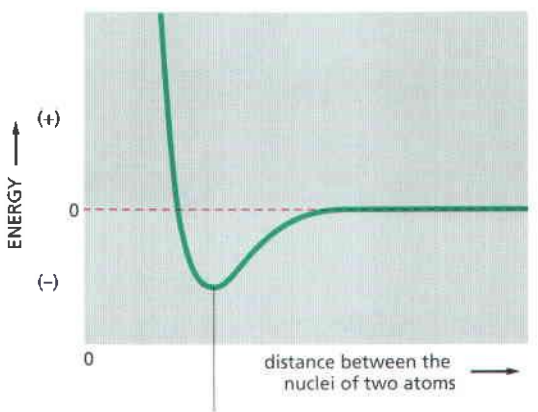


Figure 2–11 The balance of van der Waals forces between two atoms. As the nuclei of two atoms approach each other, they initially show a weak bonding interaction due to their fluctuating electric charges. However, the same atoms will strongly repel each other if they are brought too close together. The balance of these van der Waals attractive and repulsive forces occurs at the indicated energy minimum. This minimum determines the contact distance between any two noncovalently bonded atoms; this distance is the sum of their van der Waals radii. By definition, zero energy (indicated by the dotted *red* line) is the energy when the two nuclei are at infinite separation.

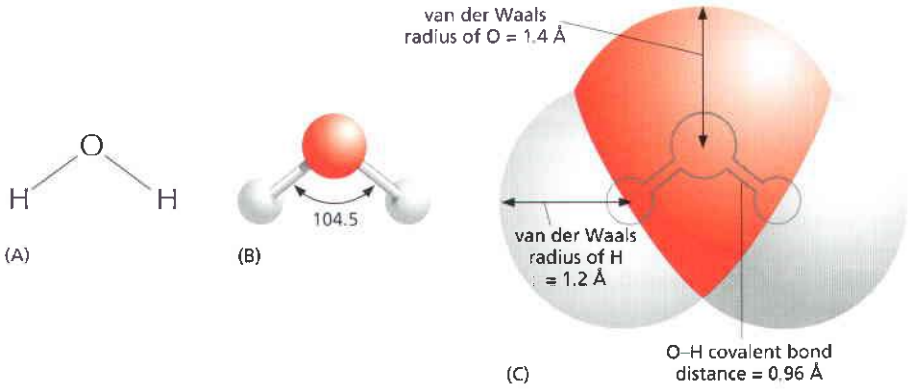


Figure 2–12 Three representations of a water molecule. (A) The usual line drawing of the structural formula, in which each atom is indicated by its standard symbol, and each line represents a covalent bond joining two atoms. (B) A ball-and-stick model, in which atoms are represented by spheres of arbitrary diameter, connected by sticks representing covalent bonds. Unlike (A), bond angles are accurately represented in this type of model (see also Figure 2–8). (C) A space-filling model, in which both bond geometry and van der Waals radii are accurately represented.

hydrogen bonds that link water molecules together that water is a liquid at room temperature, with a high boiling point and high surface tension—rather than a gas.

Molecules, such as alcohols, that contain polar bonds and that can form hydrogen bonds with water dissolve readily in water. Molecules carrying plus or minus charges (ions) likewise interact favorably with water. Such molecules are termed **hydrophilic**, meaning that they are water-loving. A large proportion of the molecules in the aqueous environment of a cell necessarily fall into this category, including sugars, DNA, RNA, and most proteins. **Hydrophobic** (water-hating) molecules, by contrast, are uncharged and form few or no hydrogen bonds, and so do not dissolve in water. Hydrocarbons are an important example (see Panel 2–1, pp. 106–107). In these molecules the H atoms are covalently linked to C atoms by a largely nonpolar bond. Because the H atoms have almost no net positive charge, they cannot form effective hydrogen bonds to other molecules. This makes the hydrocarbon as a whole hydrophobic—a property that is exploited in cells, whose membranes are constructed from molecules that have long hydrocarbon tails, as we shall see in Chapter 10.

Some Polar Molecules Are Acids and Bases

One of the simplest kinds of chemical reaction, and one that has profound significance in cells, takes place when a molecule containing a highly polar covalent bond between a hydrogen and a second atom dissolves in water. The hydrogen atom in such a molecule has largely given up its electron to the companion atom and so resembles an almost naked positively charged hydrogen nucleus—in other words, a **proton (H^+)**. When water molecules surround the polar molecule, the proton is attracted to the partial negative charge on the O atom of an adjacent water molecule and can dissociate from its original partner to associate instead with the oxygen atoms of the water molecule to generate a **hydronium ion (H_3O^+)** (Figure 2–13A). The reverse reaction also takes place very readily, so one has to imagine an equilibrium state in which billions of protons are constantly flitting to and fro from one molecule in the solution to another.

The same type of reaction takes place in a solution of pure water itself. As illustrated in Figure 2–13B, water molecules are constantly exchanging protons with each other. As a result, pure water contains an equal, very low concentration of H_3O^+ and OH^- ions, both being present at 10^{-7} M. (The concentration of H_2O in pure water is 55.5 M.)

Substances that release protons to form H_3O^+ when they dissolve in water are termed **acids**. The higher the concentration of H_3O^+, the more acidic the solution. As H_3O^+ rises, the concentration of OH^- falls, according to the equilibrium equation for water: $[H_3O^+][OH^-] = 1.0 \times 10^{-14}$, where square brackets denote molar concentrations to be multiplied. By tradition, the H_3O^+ concentration is usually referred to as the H^+ concentration, even though nearly all H^+ in an aqueous solution is present as H_3O^+. To avoid the use of unwieldy numbers, the concentration of H^+ is expressed using a logarithmic scale called the **pH scale**, as illustrated in Panel 2–2 (pp. 108–109). Pure water has a pH of 7.0, and is neutral—that is, neither acidic (pH < 7.0) nor basic (pH > 7.0).

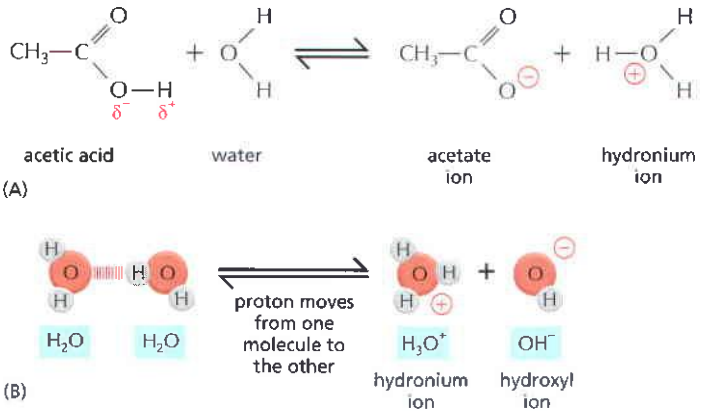


Figure 2–13 Acids in water. (A) The reaction that takes place when a molecule of acetic acid dissolves in water. (B) Water molecules are continuously exchanging protons with each other to form hydronium and hydroxyl ions. These ions in turn rapidly recombine to form water molecules.

Because the proton of a hydronium ion can be passed readily to many types of molecules in cells, altering their character, the concentration of H_3O^+ inside a cell (the acidity) must be closely regulated. The interior of a cell is kept close to neutrality, and it is buffered by the presence of many chemical groups that can take up and release protons near pH 7.

The opposite of an acid is a **base**. Just as the defining property of an acid is that it donates protons to a water molecule so as to raise the concentration of H_3O^+ ions, the defining property of a base is that it accepts protons so as to lower the concentration of H_3O^+ ions, and thereby raise the concentration of hydroxyl ions (OH^-). A base can either combine with protons directly or form hydroxyl ions that immediately combine with protons to produce H_2O. Thus sodium hydroxide (NaOH) is basic (or *alkaline*) because it dissociates in aqueous solution to form Na^+ ions and OH^- ions. Other bases, especially important in living cells, contain NH_2 groups. These groups directly take up a proton from water: $-NH_2 + H_2O \rightarrow -NH_3^+ + OH^-$.

All molecules that accept protons from water will do so most readily when the concentration of H_3O^+ is high (acidic solutions). Likewise, molecules that can give up protons do so more readily if the concentration of H_3O^+ in solution is low (basic solutions), and they will tend to receive them back if this concentration is high.

Four Types of Noncovalent Attractions Help Bring Molecules Together in Cells

In aqueous solutions, covalent bonds are 10–100 times stronger than the other attractive forces between atoms, allowing their connections to define the boundaries of one molecule from another. But much of biology depends on the specific binding of different molecules to each other. This binding is mediated by a group of noncovalent attractions that are individually quite weak, but whose energies can sum to create an effective force between two separate molecules. We have previously introduced three of these attractive forces: electrostatic attractions (ionic bonds), hydrogen bonds, and van der Waals attractions. Table 2–1 compares the strengths of these three types of *noncovalent bonds* with that of a typical covalent bond, both in the presence and in the

Table 2–1 Covalent and Noncovalent Chemical Bonds

BOND TYPE		LENGTH (nm)	STRENGTH (kcal/mole) IN VACUUM	IN WATER
Covalent		0.15	90	90
Noncovalent:	ionic*	0.25	80	3
	hydrogen	0.30	4	1
	van der Waals attraction (per atom)	0.35	0.1	0.1

*An ionic bond is an electrostatic attraction between two fully charged atoms.

absence of water. Because of their fundamental importance in all biological systems, we summarize their properties here:

- **Electrostatic attractions**. These result from the attractive forces between oppositely charged atoms. Electrostatic attractions are quite strong in the absence of water. They readily form between permanent dipoles, but are greatest when the two atoms involved are fully charged (*ionic bonds*). However, the polar water molecules cluster around both fully charged ions and polar molecules that contain permanent dipoles (Figure 2–14). This greatly reduces the attractiveness of these charged species for each other in most biological settings.
- **Hydrogen bonds**. The structure of a typical hydrogen bond is illustrated in Figure 2–15. This bond represents a special form of polar interaction in which an electropositive hydrogen atom is partially shared by two electronegative atoms. Its hydrogen can be viewed as a proton that has partially dissociated from a donor atom, allowing it to be shared by a second acceptor atom. Unlike a typical electrostatic interaction, this bond is highly directional—being strongest when a straight line can be drawn between all three of the involved atoms. As already discussed, water weakens these bonds by forming competing hydrogen-bond interactions with the involved molecules.
- **van der Waals attractions**. The electron cloud around any nonpolar atom will fluctuate, producing a flickering dipole. Such dipoles will transiently induce an oppositely polarized flickering dipole in a nearby atom. This interaction generates a very weak attraction between atoms. But since many atoms can be simultaneously in contact when two surfaces fit closely, the net result is often significant. Water does not weaken these so-called van der Waals attractions.

The fourth effect that often brings molecules together in water is not, strictly speaking, a bond at all. However, a very important **hydrophobic force** is caused by a pushing of nonpolar surfaces out of the hydrogen-bonded water network, where they would otherwise physically interfere with the highly favorable interactions between water molecules. Bringing any two nonpolar surfaces together reduces their contact with water; in this sense, the force is nonspecific. Nevertheless, we shall see in Chapter 3 that hydrophobic forces are central to the proper folding of protein molecules.

Panel 2–3 provides an overview of the four types of attractions just described. And Figure 2–16 illustrates schematically how many such interactions can sum to hold together the matching surfaces of two macromolecules, even though each interaction by itself would be much too weak to be effective in the face of thermal motions.

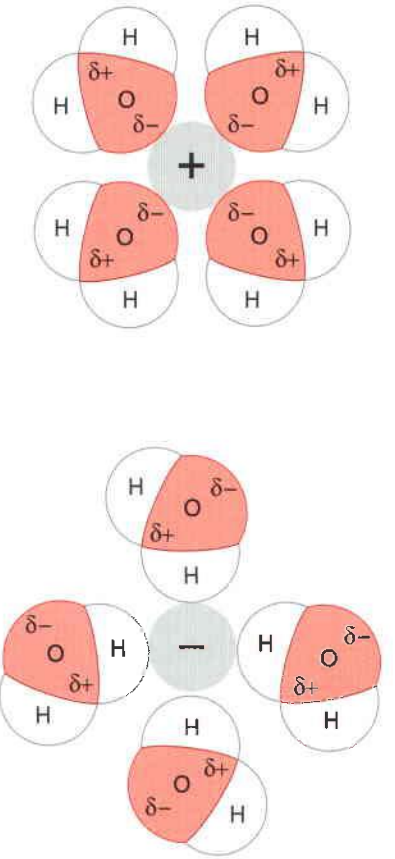


Figure 2–14 **How the dipoles on water molecules orient to reduce the affinity of oppositely charged ions or polar groups for each other.**

A Cell Is Formed from Carbon Compounds

Having looked at the ways atoms combine into small molecules and how these molecules behave in an aqueous environment, we now examine the main classes of small molecules found in cells and their biological roles. We shall see that a few basic categories of molecules, formed from a handful of different elements, give rise to all the extraordinary richness of form and behavior shown by living things.

If we disregard water and inorganic ions such as potassium, nearly all the molecules in a cell are based on carbon. Carbon is outstanding among all the elements in its ability to form large molecules; silicon is a poor second. Because it is small and has four electrons and four vacancies in its outermost shell, a carbon atom can form four covalent bonds with other atoms. Most important, one carbon atom can join to other carbon atoms through highly stable covalent C–C

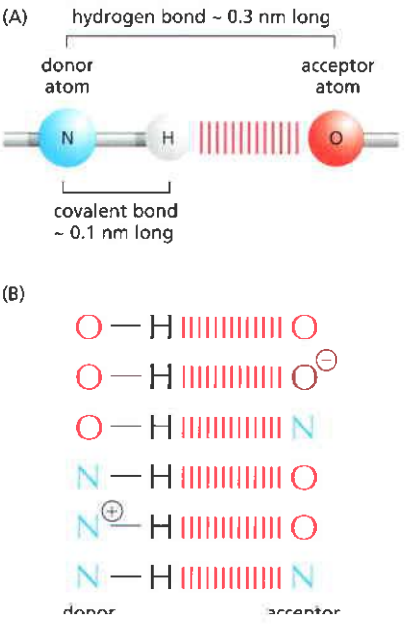


Figure 2–15 **Hydrogen bonds.** (A) Ball-and-stick model of a typical hydrogen bond. The distance between the hydrogen and the oxygen atom here is less than the sum of their van der Waals radii, indicating a partial sharing of electrons. (B) The most common hydrogen bonds in cells.

bonds to form chains and rings and hence generate large and complex molecules with no obvious upper limit to their size (see Panel 2–1, pp. 106–107). The small and large carbon compounds made by cells are called *organic molecules.*

Certain combinations of atoms, such as the methyl ($-CH_3$), hydroxyl (–OH), carboxyl (–COOH), carbonyl (–C=O), phosphate ($-PO_3^{2-}$), sulfhydryl (–SH), and amino ($-NH_2$) groups, occur repeatedly in organic molecules. Each such **chemical group** has distinct chemical and physical properties that influence the behavior of the molecule in which the group occurs. The most common chemical groups and some of their properties are summarized in Panel 2–1, pp. 106–107.

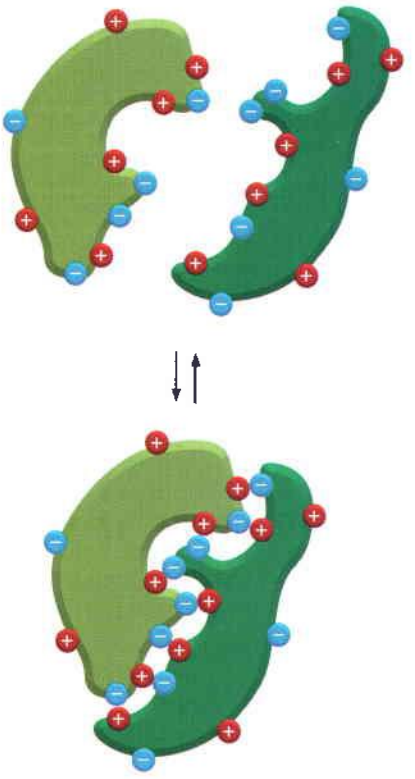


Figure 2–16 Schematic indicating how two macromolecules with complementary surfaces can bind tightly to one another through noncovalent interactions.

Cells Contain Four Major Families of Small Organic Molecules

The small organic molecules of the cell are carbon-based compounds that have molecular weights in the range 100–1000 and contain up to 30 or so carbon atoms. They are usually found free in solution and have many different fates. Some are used as *monomer* subunits to construct the giant polymeric *macromolecules*—the proteins, nucleic acids, and large polysaccharides—of the cell. Others act as energy sources and are broken down and transformed into other small molecules in a maze of intracellular metabolic pathways. Many small molecules have more than one role in the cell—for example, acting both as a potential subunit for a macromolecule and as an energy source. Small organic molecules are much less abundant than the organic macromolecules, accounting for only about one-tenth of the total mass of organic matter in a cell (Table 2–2). As a rough guess, there may be a thousand different kinds of these small molecules in a typical cell.

All organic molecules are synthesized from and are broken down into the same set of simple compounds. Both their synthesis and their breakdown occur through sequences of limited chemical changes that follow definite rules. As a consequence, the compounds in a cell are chemically related and most can be classified into a few distinct families. Broadly speaking, cells contain four major families of small organic molecules: the *sugars*, the *fatty acids*, the *amino acids*, and the *nucleotides* (Figure 2–17). Although many compounds present in cells do not fit into these categories, these four families of small organic molecules, together with the macromolecules made by linking them into long chains, account for a large fraction of cell mass (see Table 2–2).

Sugars Provide an Energy Source for Cells and Are the Subunits of Polysaccharides

The simplest **sugars**—the *monosaccharides*—are compounds with the general formula $(CH_2O)_n$, where n is usually 3, 4, 5, 6, 7, or 8. Sugars, and the molecules made from them, are also called *carbohydrates* because of this simple formula. Glucose, for example, has the formula $C_6H_{12}O_6$ (Figure 2–18). The formula, however, does not fully define the molecule: the same set of carbons, hydrogens, and

Table 2–2 The Types of Molecules That Form a Bacterial Cell

	PERCENT OF TOTAL CELL WEIGHT	NUMBER OF TYPES OF EACH MOLECULE
Water	70	1
Inorganic ions	1	20
Sugars and precursors	1	250
Amino acids and precursors	0.4	100
Nucleotides and precursors	0.4	100
Fatty acids and precursors	1	50
Other small molecules	0.2	~300
Macromolecules (proteins, nucleic acids, and polysaccharides)	26	~3000

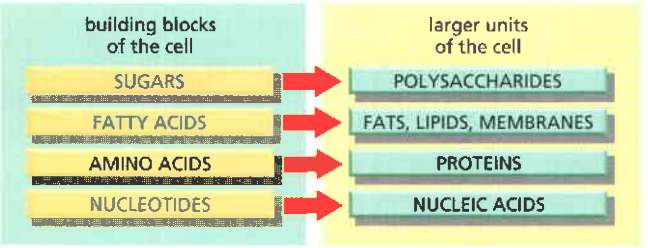


Figure 2–17 The four main families of small organic molecules in cells. These small molecules form the monomeric building blocks, or subunits, for most of the macromolecules and other assemblies of the cell. Some, such as the sugars and the fatty acids, are also energy sources.

oxygens can be joined together by covalent bonds in a variety of ways, creating structures with different shapes. As shown in Panel 2–4 (pp. 112–113), for example, glucose can be converted into a different sugar—mannose or galactose—simply by switching the orientations of specific OH groups relative to the rest of the molecule. Each of these sugars, moreover, can exist in either of two forms, called the D-form and the L-form, which are mirror images of each other. Sets of molecules with the same chemical formula but different structures are called *isomers*, and the subset of such molecules that are mirror-image pairs are called *optical isomers*. Isomers are widespread among organic molecules in general, and they play a major part in generating the enormous variety of sugars.

Panel 2–4 presents an outline of sugar structure and chemistry. Sugars can exist as rings or as open chains. In their open-chain form, sugars contain a number of hydroxyl groups and either one aldehyde ($_{H}{>}C{=}O$) or one ketone ($>C{=}O$) group. The aldehyde or ketone group plays a special role. First, it can react with a hydroxyl group in the same molecule to convert the molecule into a ring; in the ring form the carbon of the original aldehyde or ketone group can be recognized as the only one that is bonded to two oxygens. Second, once the ring is formed, this same carbon can become further linked, via oxygen, to one of the carbons bearing a hydroxyl group on another sugar molecule. This creates a *disaccharide* such as sucrose, which is composed of a glucose and a fructose unit. Larger sugar polymers range from the *oligosaccharides* (trisaccharides, tetrasaccharides, and so on) up to giant *polysaccharides*, which can contain thousands of monosaccharide units.

The way that sugars are linked together to form polymers illustrates some common features of biochemical bond formation. A bond is formed between an –OH group on one sugar and an –OH group on another by a **condensation reaction,** in which a molecule of water is expelled as the bond is formed (Figure 2–19). Subunits in other biological polymers, such as nucleic acids and proteins, are also linked by condensation reactions in which water is expelled. The bonds created by all of these condensation reactions can be broken by the reverse process of **hydrolysis**, in which a molecule of water is consumed (see Figure 2–19).

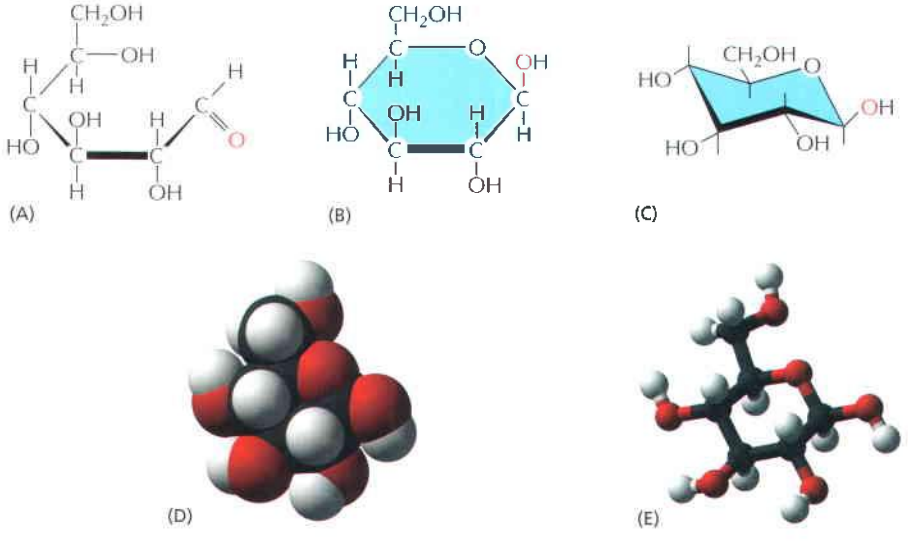


Figure 2–18 The structure of glucose, a simple sugar. As illustrated previously for water (see Figure 2–12), any molecule can be represented in several ways. In the structural formulas shown in (A), (B) and (C), the atoms are shown as chemical symbols linked together by lines representing the covalent bonds. The *thickened lines* here are used to indicate the plane of the sugar ring, in an attempt to emphasize that the –H and –OH groups are not in the same plane as the ring. (A) The open-chain form of this sugar, which is in equilibrium with the more stable cyclic or ring form in (B). (C) The chair form is an alternative way to draw the cyclic molecule that reflects the geometry more accurately than the structural formula in (B). (D) A space-filling model, which, as well as depicting the three-dimensional arrangement of the atoms, also uses the van der Waals radii to represent the surface contours of the molecule. (E) A ball-and-stick model in which the three-dimensional arrangement of the atoms in space is shown. (H, *white*; C, *black*; O, *red*; N, *blue*.)

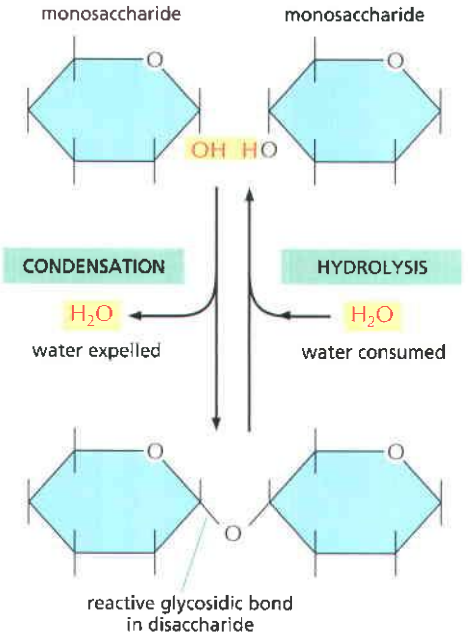


Figure 2–19 The reaction of two monosaccharides to form a disaccharide. This reaction belongs to a general category of reactions termed *condensation reactions*, in which two molecules join together as a result of the loss of a water molecule. The reverse reaction (in which water is added) is termed *hydrolysis*. Note that the reactive carbon at which the new bond is formed (on the monosaccharide on the *left* here) is the carbon joined to two oxygens as a result of sugar ring formation (see Figure 2–18). As indicated, this common type of covalent bond between two sugar molecules is known as a *glycosidic bond* (see also Figure 2–20).

Because each monosaccharide has several free hydroxyl groups that can form a link to another monosaccharide (or to some other compound), sugar polymers can be branched, and the number of possible polysaccharide structures is extremely large. Even a simple disaccharide consisting of two glucose units can exist in eleven different varieties (**Figure 2–20**), while three different hexoses ($C_6H_{12}O_6$) can join together to make several thousand trisaccharides. For this reason it is a much more complex task to determine the arrangement of sugars in a polysaccharide than to determine the nucleotide sequence of a DNA molecule, where each unit is joined to the next in exactly the same way.

The monosaccharide *glucose* is a key energy source for cells. In a series of reactions, it is broken down to smaller molecules, releasing energy that the cell can harness to do useful work, as we shall explain later. Cells use simple polysaccharides composed only of glucose units—principally *glycogen* in animals and *starch* in plants—as energy stores.

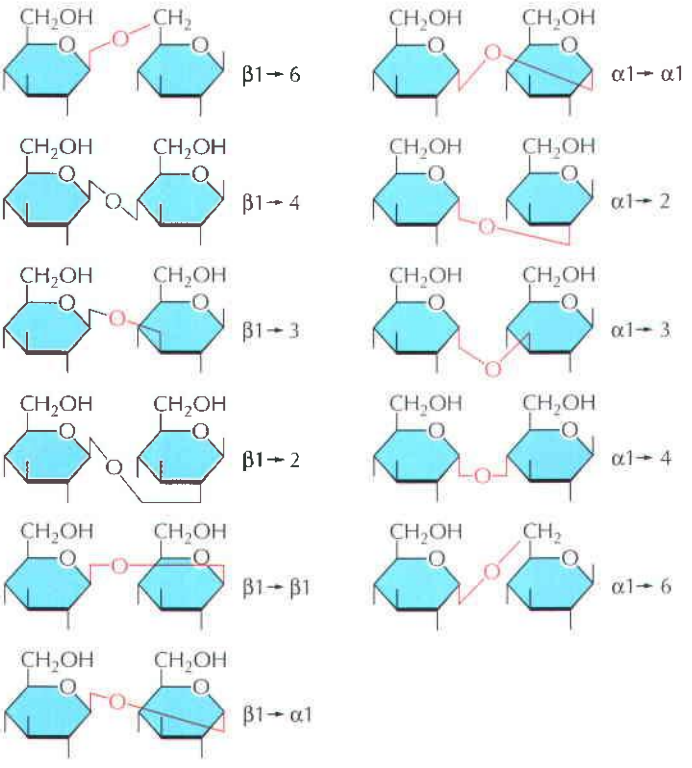


Figure 2–20 Eleven disaccharides consisting of two D-glucose units. Although these differ only in the type of linkage between the two glucose units, they are chemically distinct. Since the oligosaccharides associated with proteins and lipids may have six or more different kinds of sugar joined in both linear and branched arrangements through glycosidic bonds such as those illustrated here, the number of distinct types of oligosaccharides that can be used in cells is extremely large. For an explanation of α and β linkages, see Panel 2–4 (pp. 112–113). Short *black* lines ending "blind" indicate OH positions. (*Red* lines merely indicate disaccharide bond orientations and "corners" do not imply extra atoms.)

Sugars do not function only in the production and storage of energy. They can also be used, for example, to make mechanical supports. Thus, the most abundant organic chemical on Earth—the *cellulose* of plant cell walls—is a polysaccharide of glucose. Because the glucose–glucose linkages in cellulose differ from those in starch and glycogen, however, humans cannot digest cellulose and use its glucose. Another extraordinarily abundant organic substance, the *chitin* of insect exoskeletons and fungal cell walls, is also an indigestible polysaccharide—in this case a linear polymer of a sugar derivative called *N*-acetylglucosamine (see Panel 2–4). Other polysaccharides are the main components of slime, mucus, and gristle.

Smaller oligosaccharides can be covalently linked to proteins to form glycoproteins and to lipids to form *glycolipids*, both of which are found in cell membranes. As described in Chapter 10, most cell surfaces are clothed and decorated with glycoproteins and glycolipids in the cell membrane. The sugar side chains on these molecules are often recognized selectively by other cells. And differences between people in the details of their cell-surface sugars are the molecular basis for the different major human blood groups, termed A, B, AB, and O.

Fatty Acids Are Components of Cell Membranes, as Well as a Source of Energy

A fatty acid molecule, such as *palmitic acid*, has two chemically distinct regions (**Figure 2–21**). One is a long hydrocarbon chain, which is hydrophobic and not very reactive chemically. The other is a carboxyl (–COOH) group, which behaves as an acid (carboxylic acid): it is ionized in solution ($-COO^-$), extremely hydrophilic, and chemically reactive. Almost all the fatty acid molecules in a cell are covalently linked to other molecules by their carboxylic acid group.

The hydrocarbon tail of palmitic acid is *saturated:* it has no double bonds between carbon atoms and contains the maximum possible number of hydrogens. Stearic acid, another one of the common fatty acids in animal fat, is also saturated. Some other fatty acids, such as oleic acid, have *unsaturated* tails, with one or more double bonds along their length. The double bonds create kinks in the molecules, interfering with their ability to pack together in a solid mass. It is this that accounts for the difference between hard margarine (saturated) and liquid vegetable oils (polyunsaturated). The many different fatty acids found in cells differ only in the length of their hydrocarbon chains and the number and position of the carbon–carbon double bonds (see Panel 2–5, pp. 114–115).

Fatty acids are stored in the cytoplasm of many cells in the form of droplets of *triacylglycerol* molecules, which consist of three fatty acid chains joined to a glycerol molecule (see Panel 2–5); these molecules are the animal fats found in meat, butter, and cream, and the plant oils such as corn oil and olive oil. When required to provide energy, the fatty acid chains are released from triacylglycerols and broken down into two-carbon units. These two-carbon units are identical to those derived from the breakdown of glucose and they enter the same energy-yielding reaction pathways, as will be described later in this chapter. Triglycerides serve as a concentrated food reserve in cells, because they can be broken down to produce about six times as much usable energy, weight for weight, as glucose.

Fatty acids and their derivatives such as triacylglycerols are examples of **lipids.** Lipids comprise a loosely defined collection of biological molecules that are insoluble in water, while being soluble in fat and organic solvents such as benzene. They typically contain either long hydrocarbon chains, as in the fatty acids and isoprenes, or multiple linked rings, as in the *steroids.*

The most important function of fatty acids in cells is in the construction of cell membranes. These thin sheets enclose all cells and surround their internal organelles. They are composed largely of *phospholipids*, which are small molecules that, like triacylglycerols, are constructed mainly from fatty acids and glycerol. In phospholipids the glycerol is joined to two fatty acid chains, however, rather than to three as in triacylglycerols. The "third" site on the glycerol is linked to a hydrophilic phosphate group, which is in turn attached to a small hydrophilic compound such as choline (see Panel 2–5). Each phospholipid

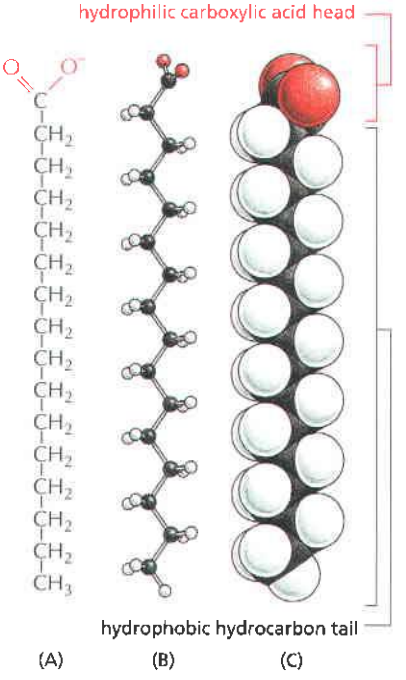


Figure 2–21 A fatty acid. A fatty acid is composed of a hydrophobic hydrocarbon chain to which is attached a hydrophilic carboxylic acid group. Palmitic acid is shown here. Different fatty acids have different hydrocarbon tails. (A) Structural formula. The carboxylic acid group is shown in its ionized form. (B) Ball-and-stick model. (C) Space-filling model.

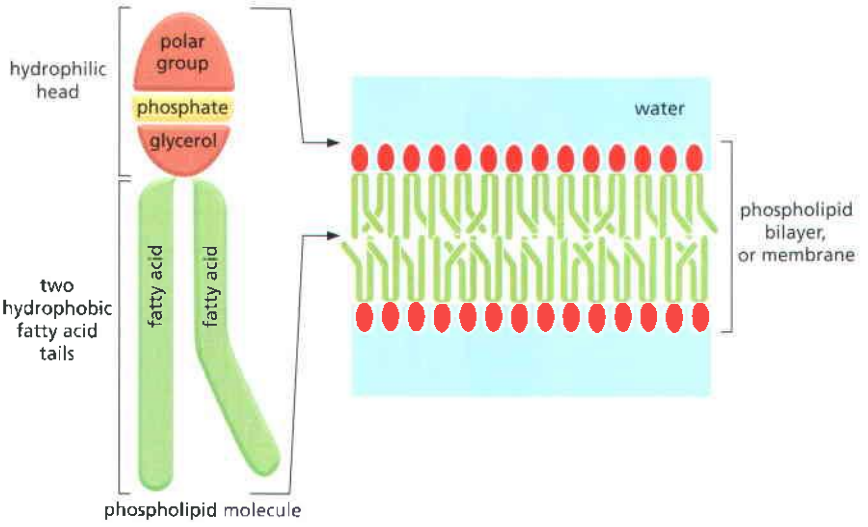


Figure 2–22 Phospholipid structure and the orientation of phospholipids in membranes. In an aqueous environment, the hydrophobic tails of phospholipids pack together to exclude water. Here they have formed a bilayer with the hydrophilic head of each phospholipid facing the water. Lipid bilayers are the basis for cell membranes, as discussed in detail in Chapter 10.

molecule, therefore, has a hydrophobic tail composed of the two fatty acid chains and a hydrophilic head, where the phosphate is located. This gives them different physical and chemical properties from triacylglycerols, which are predominantly hydrophobic. Molecules such as phospholipids, with both hydrophobic and hydrophilic regions, are termed *amphiphilic*.

The membrane-forming property of phospholipids results from their amphiphilic nature. Phospholipids will spread over the surface of water to form a monolayer of phospholipid molecules, with the hydrophobic tails facing the air and the hydrophilic heads in contact with the water. Two such molecular layers can readily combine tail-to-tail in water to make a phospholipid sandwich, or **lipid bilayer**. This bilayer is the structural basis of all cell membranes (**Figure 2–22**).

Amino Acids Are the Subunits of Proteins

Amino acids are a varied class of molecules with one defining property: they all possess a carboxylic acid group and an amino group, both linked to a single carbon atom called the α-carbon (**Figure 2–23**). Their chemical variety comes from the side chain that is also attached to the α-carbon. The importance of amino acids to the cell comes from their role in making **proteins**, which are polymers of amino acids joined head-to-tail in a long chain that is then folded into a three-dimensional structure unique to each type of protein. The covalent linkage between two adjacent amino acids in a protein chain forms an amide (see Panel 2–1), and it is called a **peptide bond**; the chain of amino acids is also known as a *polypeptide* (**Figure 2–24**). Regardless of the specific amino acids from which it is made, the polypeptide has an amino (NH_2) group at one end (its *N-terminus*) and a carboxyl (COOH) group at its other end (its *C-terminus*). This gives it a definite directionality—a structural (as opposed to an electrical) polarity.

Each of the 20 amino acids found commonly in proteins has a different side chain attached to the α-carbon atom (see Panel 3–1, pp. 128–129). All organisms,

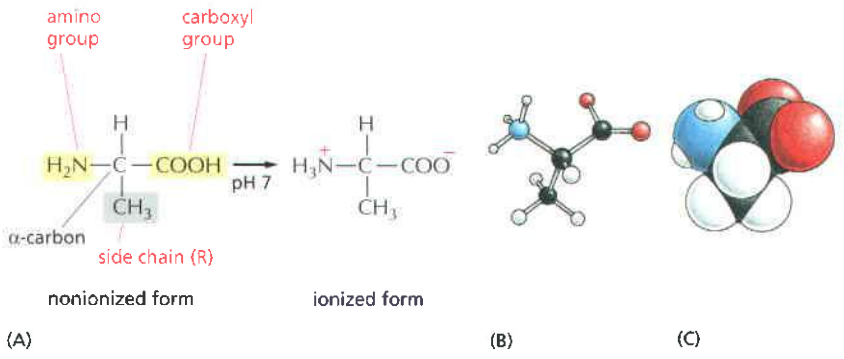


Figure 2–23 The amino acid alanine. (A) In the cell, where the pH is close to 7, the free amino acid exists in its ionized form; but when it is incorporated into a polypeptide chain, the charges on the amino and carboxyl groups disappear. (B) A ball-and-stick model and (C) a space-filling model of alanine (H, *white;* C, *black;* O, *red;* N, *blue*).

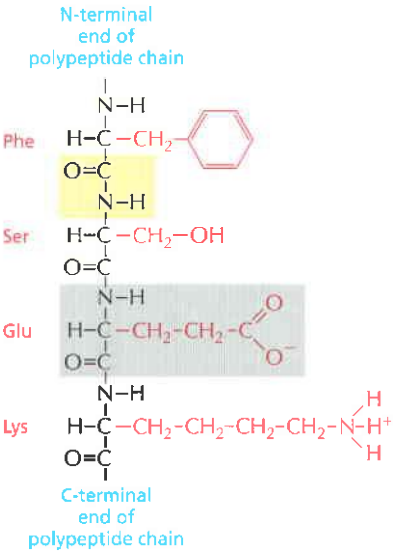


Figure 2–24 A small part of a protein molecule. The four amino acids shown are linked together by three peptide bonds, one of which is highlighted in *yellow*. One of the amino acids is shaded in *gray*. The amino acid side chains are shown in *red*. The two ends of a polypeptide chain are chemically distinct. One end, the N-terminus, terminates in an amino group, and the other, the C-terminus, in a carboxyl group. The sequence is always read from the N-terminal end; hence this sequence is Phe–Ser–Glu–Lys.

whether bacteria, archea, plants, or animals, have proteins made of the same 20 amino acids. How this precise set of 20 came to be chosen is one of the mysteries of the evolution of life; there is no obvious chemical reason why other amino acids could not have served just as well. But once the choice was established, it could not be changed; too much depended on it.

Like sugars, all amino acids, except glycine, exist as optical isomers in D- and L-forms (see Panel 3–1). But only L-forms are ever found in proteins (although D-amino acids occur as part of bacterial cell walls and in some antibiotics). The origin of this exclusive use of L-amino acids to make proteins is another evolutionary mystery.

The chemical versatility of the 20 amino acids is essential to the function of proteins. Five of the 20 amino acids have side chains that can form ions in neutral aqueous solution and thereby can carry a charge (**Figure 2–25**). The others are uncharged; some are polar and hydrophilic, and some are nonpolar and hydrophobic. As we discuss in Chapter 3, the properties of the amino acid side chains underlie the diverse and sophisticated functions of proteins.

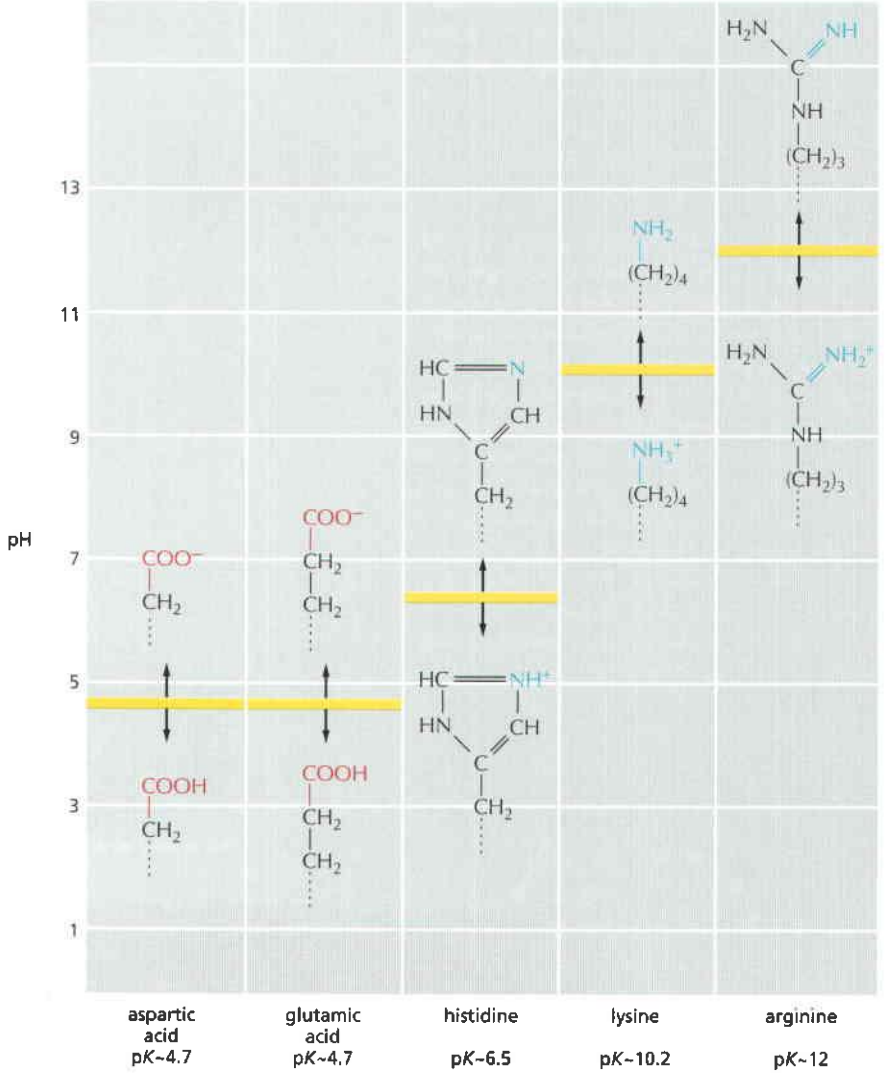


Figure 2–25 The charge on amino acid side chains depends on the pH. The five different side chains that can carry a charge are shown. Carboxylic acids can readily lose H^+ in aqueous solution to form a negatively charged ion, which is denoted by the suffix "-ate," as in aspart*ate* or glutam*ate*. A comparable situation exists for amines, which in aqueous solution can take up H^+ to form a positively charged ion (which does not have a special name). These reactions are rapidly reversible, and the amounts of the two forms, charged and uncharged, depend on the pH of the solution. At a high pH, carboxylic acids tend to be charged and amines uncharged. At a low pH, the opposite is true—the carboxylic acids are uncharged and amines are charged. The pH at which exactly *half* of the carboxylic acid or amine residues are charged is known as the p*K* of that amino acid side chain (indicated by *yellow* stripe).

In the cell the pH is close to 7, and almost all carboxylic acids and amines are in their fully charged form.

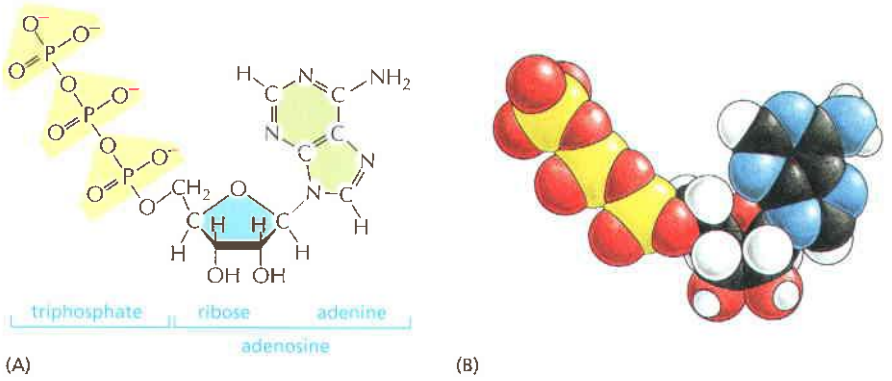


Figure 2–26 Chemical structure of adenosine triphosphate (ATP). (A) Structural formula. (B) Space-filling model. In (B) the colors of the atoms are C, *black;* N, *blue;* H, *white;* O, *red;* and P, *yellow.*

Nucleotides Are the Subunits of DNA and RNA

A **nucleotide** is a molecule made up of a nitrogen-containing ring compound linked to a five-carbon sugar, which in turn carries one or more phosphate groups (Panel 2–6, pp. 116–117). The five-carbon sugar can be either ribose or deoxyribose. Nucleotides containing ribose are known as ribonucleotides, and those containing deoxyribose as deoxyribonucleotides. The nitrogen-containing rings are generally referred to as *bases* for historical reasons: under acidic conditions they can each bind an H^+ (proton) and thereby increase the concentration of OH^- ions in aqueous solution. There is a strong family resemblance between the different bases. *Cytosine (C), thymine (T),* and *uracil (U)* are called pyrimidines because they all derive from a six-membered pyrimidine ring; *guanine (G)* and *adenine (A)* are *purine* compounds, and they have a second, five-membered ring fused to the six-membered ring. Each nucleotide is named for the base it contains (see Panel 2–6).

Nucleotides can act as short-term carriers of chemical energy. Above all others, the ribonucleotide **adenosine triphosphate**, or **ATP** (Figure 2–26), transfers energy in hundreds of different cell reactions. ATP is formed through reactions that are driven by the energy released by the oxidative breakdown of foodstuffs. Its three phosphates are linked in series by two *phosphoanhydride bonds*, whose rupture releases large amounts of useful energy. The terminal phosphate group in particular is frequently split off by hydrolysis, often transferring a phosphate to other molecules and releasing energy that drives energy-requiring biosynthetic reactions (Figure 2–27). Other nucleotide derivatives are carriers for the transfer of other chemical groups, as will be described later.

The most fundamental role of nucleotides in the cell, however, is in the storage and retrieval of biological information. Nucleotides serve as building blocks for the construction of *nucleic acids*—long polymers in which nucleotide subunits are covalently linked by the formation of a **phosphodiester bond** between the

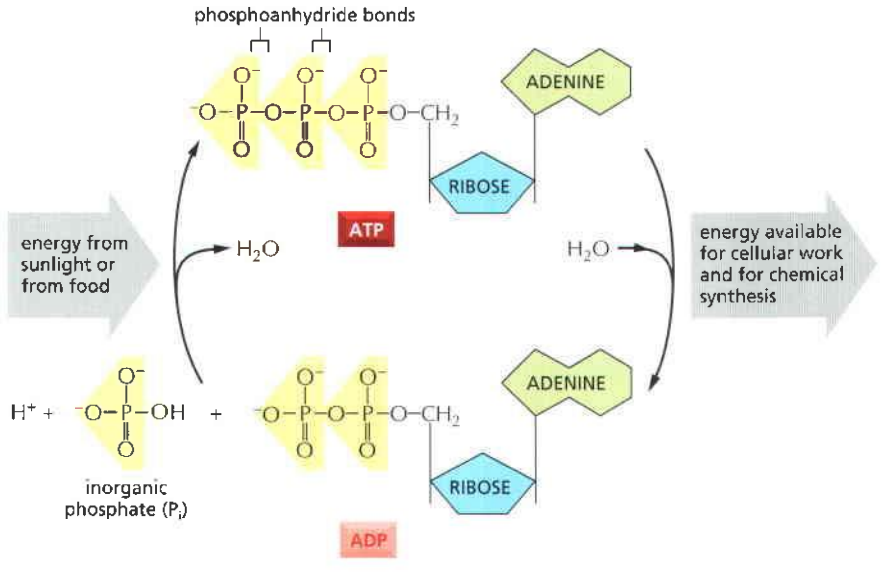


Figure 2–27 The ATP molecule serves as an energy carrier in cells. The energy-requiring formation of ATP from ADP and inorganic phosphate is coupled to the energy-yielding oxidation of foodstuffs (in animal cells, fungi, and some bacteria) or to the capture of light energy (in plant cells and some bacteria). The hydrolysis of this ATP back to ADP and inorganic phosphate in turn provides the energy to drive many cell reactions.

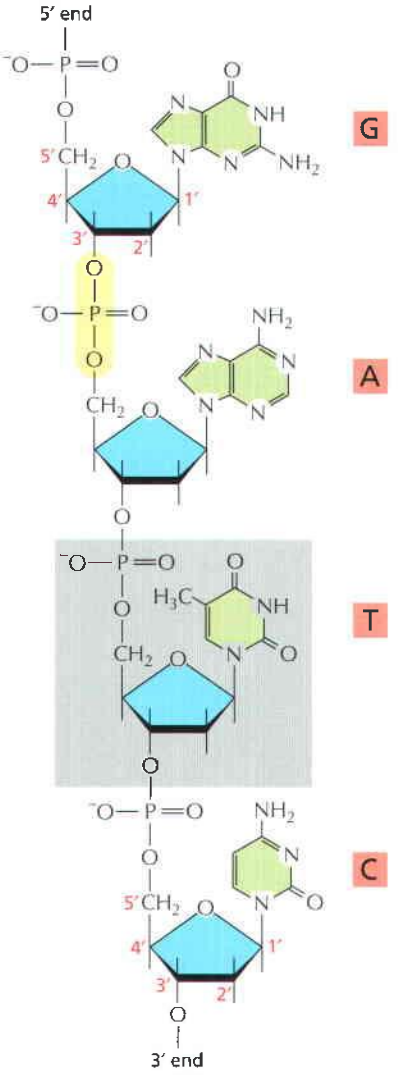


Figure 2–28 A small part of one chain of a deoxyribonucleic acid (DNA) molecule. Four nucleotides are shown. One of the phosphodiester bonds that links adjacent nucleotide residues is highlighted in *yellow*, and one of the nucleotides is shaded in *gray*. Nucleotides are linked together by a phosphodiester linkage between specific carbon atoms of the ribose, known as the 5′ and 3′ atoms. For this reason, one end of a polynucleotide chain, the *5′ end*, will have a free phosphate group and the other, the *3′ end*, a free hydroxyl group. The linear sequence of nucleotides in a polynucleotide chain is commonly abbreviated by a one-letter code, and the sequence is always read from the 5′ end. In the example illustrated the sequence is G–A–T–C.

phosphate group attached to the sugar of one nucleotide and a hydroxyl group on the sugar of the next nucleotide (**Figure 2–28**). Nucleic acid chains are synthesized from energy-rich nucleoside triphosphates by a condensation reaction that releases inorganic pyrophosphate during phosphodiester bond formation.

There are two main types of nucleic acids, differing in the type of sugar in their sugar-phosphate backbone. Those based on the sugar *ribose* are known as **ribonucleic acids**, or **RNA**, and normally contain the bases A, G, C, and U. Those based on *deoxyribose* (in which the hydroxyl at the 2′ position of the ribose carbon ring is replaced by a hydrogen are known as **deoxyribonucleic acids**, or **DNA**, and contain the bases A, G, C, and T (T is chemically similar to the U in RNA, merely adding the methyl group on the pyrimidine ring; see Panel 2–6). RNA usually occurs in cells as a single polynucleotide chain, but DNA is virtually always a double-stranded molecule—a DNA double helix composed of two polynucleotide chains running antiparallel to each other and held together by hydrogen-bonding between the bases of the two chains.

The linear sequence of nucleotides in a DNA or an RNA encodes the genetic information of the cell. The ability of the bases in different nucleic acid molecules to recognize and pair with each other by hydrogen-bonding (called *base-pairing*)—G with C, and A with either T or U—underlies all of heredity and evolution, as explained in Chapter 4.

The Chemistry of Cells Is Dominated by Macromolecules with Remarkable Properties

By weight, macromolecules are the most abundant carbon-containing molecules in a living cell (**Figure 2–29** and **Table 2–3**). They are the principal building blocks from which a cell is constructed and also the components that confer the most distinctive properties of living things. The macromolecules in cells are polymers that are constructed by covalently linking small organic molecules (called *monomers)* into long chains (**Figure 2–30**). Yet they have remarkable properties that could not have been predicted from their simple constituents.

Proteins are especially abundant and versatile. They perform thousands of distinct functions in cells. Many proteins serve as *enzymes*, the catalysts that

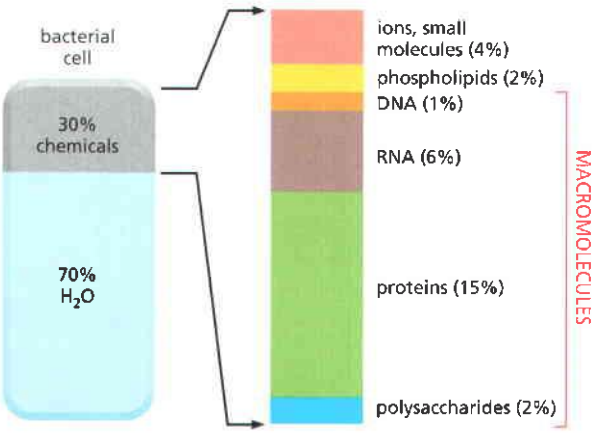


Figure 2–29 Macromolecules are abundant in cells. The approximate composition of a bacterial cell is shown by weight. The composition of an animal cell is similar (see Table 2–3).

Table 2–3 Approximate Chemical Compositions of a Typical Bacterium and a Typical Mammalian Cell

COMPONENT	PERCENT OF TOTAL CELL WEIGHT	
	E. COLI BACTERIUM	MAMMALIAN CELL
H_2O	70	70
Inorganic ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, etc.)	1	1
Miscellaneous small metabolites	3	3
Proteins	15	18
RNA	6	1.1
DNA	1	0.25
Phospholipids	2	3
Other lipids	–	2
Polysaccharides	2	2
Total cell volume	2×10^{-12} cm^3	4×10^{-9} cm^3
Relative cell volume	1	2000

Proteins, polysaccharides, DNA, and RNA are macromolecules. Lipids are not generally classed as macromolecules even though they share some of their features; for example, most are synthesized as linear polymers of a smaller molecule (the acetyl group on acetyl CoA), and they self-assemble into larger structures (membranes). Note that water and protein comprise most of the mass of both mammalian and bacterial cells.

direct the many covalent bond-making and bond-breaking reactions that the cell needs. Enzymes catalyze all of the reactions whereby cells extract energy from food molecules, for example, and an enzyme called ribulose bisphosphate carboxylase helps to convert CO_2 to sugars in photosynthetic organisms, producing most of the organic matter needed for life on Earth. Other proteins are used to build structural components, such as tubulin, a protein that self-assembles to make the cell's long microtubules, or histones, proteins that compact the DNA in chromosomes. Yet other proteins act as molecular motors to produce force and movement, as in the case of myosin in muscle. Proteins perform many other functions, and we shall examine the molecular basis for many of them later in this book. Here we identify some general principles of macromolecular chemistry that make such functions possible.

Although the chemical reactions for adding subunits to each polymer are different in detail for proteins, nucleic acids, and polysaccharides, they share important features. Each polymer grows by the addition of a monomer onto the end of a growing polymer chain in a *condensation reaction*, in which a molecule of water is lost with each subunit added (see Figure 2–19). The stepwise polymerization of monomers into a long chain is a simple way to manufacture a large, complex molecule, since the subunits are added by the same reaction performed over and over again by the same set of enzymes. In a sense, the process resembles the repetitive operation of a machine in a factory—except in one crucial respect. Apart from some of the polysaccharides, most macromolecules are made from a set of monomers that are slightly different from one another—for example, the 20 different amino acids from which proteins are made. It is critical to life that the polymer chain is not assembled at random from these subunits; instead the subunits are added in a particular order, or *sequence*. The elaborate mechanisms that allow this to be accomplished by enzymes are described in detail in Chapters 5 and 6.

Noncovalent Bonds Specify Both the Precise Shape of a Macromolecule and its Binding to Other Molecules

Most of the covalent bonds in a macromolecule allow rotation of the atoms they join, giving the polymer chain great flexibility. In principle, this allows a macromolecule to adopt an almost unlimited number of shapes, or *conformations*, as

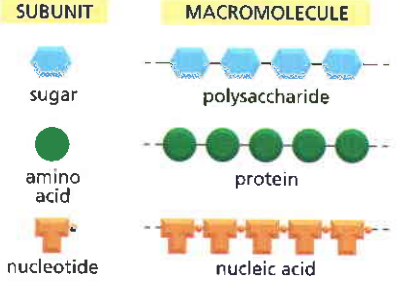


Figure 2–30 Three families of macromolecules. Each is a polymer formed from small molecules (called monomers) linked together by covalent bonds.

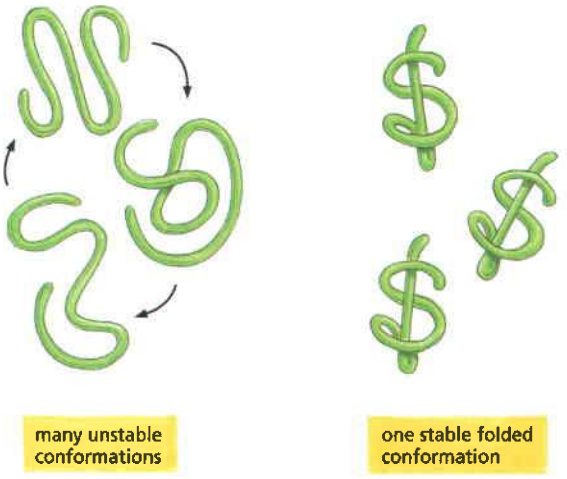


Figure 2–31 Most proteins and many RNA molecules fold into only one stable conformation. If the noncovalent bonds maintaining this stable conformation are disrupted, the molecule becomes a flexible chain that usually has no biological value.

random thermal energy causes the polymer chain to writhe and rotate. However, the shapes of most biological macromolecules are highly constrained because of the many weak *noncovalent bonds* that form between different parts of the same molecule. If these noncovalent bonds are formed in sufficient numbers, the polymer chain can strongly prefer one particular conformation, determined by the linear sequence of monomers in its chain. Most protein molecules and many of the small RNA molecules found in cells fold tightly into one highly preferred conformation in this way (**Figure 2–31**).

The four types of noncovalent interactions important in biological molecules were described earlier, and they are reviewed in Panel 2–3 (pp. 110–111). Although individually very weak, these interactions cooperate to fold biological macromolecules into unique shapes. In addition, they can also add up to create a strong attraction between two different molecules when these molecules fit together very closely, like a hand in a glove. This form of molecular interaction provides for great specificity, inasmuch as the multipoint contacts required for strong binding make it possible for a macromolecule to select out—through binding—just one of the many thousands of other types of molecules present inside a cell. Moreover, because the strength of the binding depends on the number of noncovalent bonds that are formed, interactions of almost any affinity are possible—allowing rapid dissociation when necessary.

Binding of this type underlies all biological catalysis, making it possible for proteins to function as enzymes. Noncovalent interactions also allow macromolecules to be used as building blocks for the formation of larger structures. In cells, macromolecules often bind together into large complexes, thereby forming intricate machines with multiple moving parts that perform such complex tasks as DNA replication and protein synthesis (**Figure 2–32**).

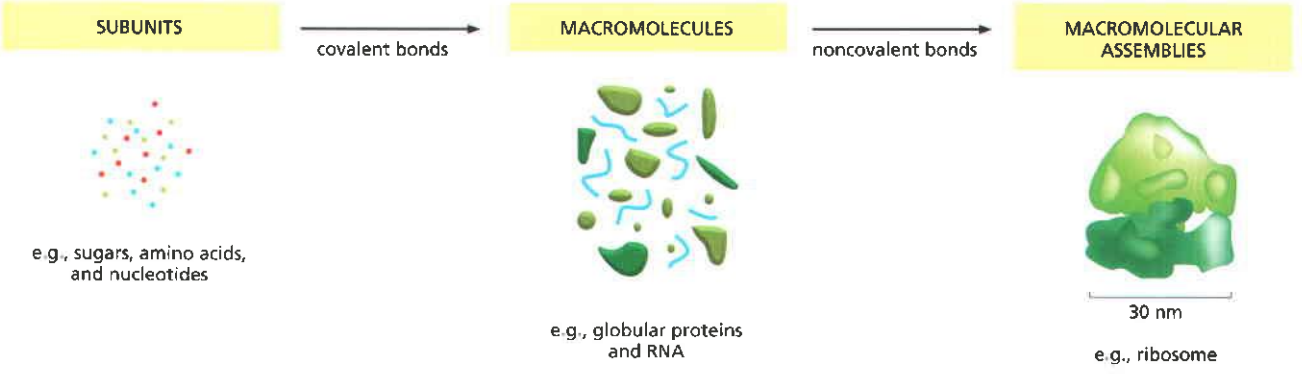


Figure 2–32 Small molecules, proteins, and a ribosome drawn approximately to scale. Ribosomes are a central part of the machinery that the cell uses to make proteins: each ribosome is formed as a complex of about 90 macromolecules (protein and RNA molecules).

Summary

Living organisms are autonomous, self-propagating chemical systems. They are made from a distinctive and restricted set of small carbon-based molecules that are essentially the same for every living species. Each of these molecules is composed of a small set of atoms linked to each other in a precise configuration through covalent bonds. The main categories are sugars, fatty acids, amino acids, and nucleotides. Sugars are a primary source of chemical energy for cells and can be incorporated into polysaccharides for energy storage. Fatty acids are also important for energy storage, but their most critical function is in the formation of cell membranes. Polymers consisting of amino acids constitute the remarkably diverse and versatile macromolecules known as proteins. Nucleotides play a central part in energy transfer. They are also the subunits for the informational macromolecules, RNA and DNA.

Most of the dry mass of a cell consists of macromolecules that have been produced as linear polymers of amino acids (proteins) or nucleotides (DNA and RNA), covalently linked to each other in an exact order. Most of the protein molecules and many of the RNAs fold into a unique conformation that depends on their sequence of subunits. This folding process creates unique surfaces, and it depends on a large set of weak attractions produced by noncovalent forces between atoms. These forces are of four types: electrostatic attractions, hydrogen bonds, van der Waals attractions, and an interaction between nonpolar groups caused by their hydrophobic expulsion from water. The same set of weak forces governs the specific binding of other molecules to macromolecules, making possible the myriad associations between biological molecules that produce the structure and the chemistry of a cell.

CATALYSIS AND THE USE OF ENERGY BY CELLS

One property of living things above all makes them seem almost miraculously different from nonliving matter: they create and maintain order, in a universe that is tending always to greater disorder (**Figure 2–33**). To create this order, the cells in a living organism must perform a never-ending stream of chemical reactions. In some of these reactions, small organic molecules—amino acids, sugars, nucleotides, and lipids—are being taken apart or modified to supply the many other small molecules that the cell requires. In other reactions, these small molecules are being used to construct an enormously diverse range of proteins, nucleic acids, and other macromolecules that endow living systems with all of their most distinctive properties. Each cell can be viewed as a tiny chemical factory, performing many millions of reactions every second.

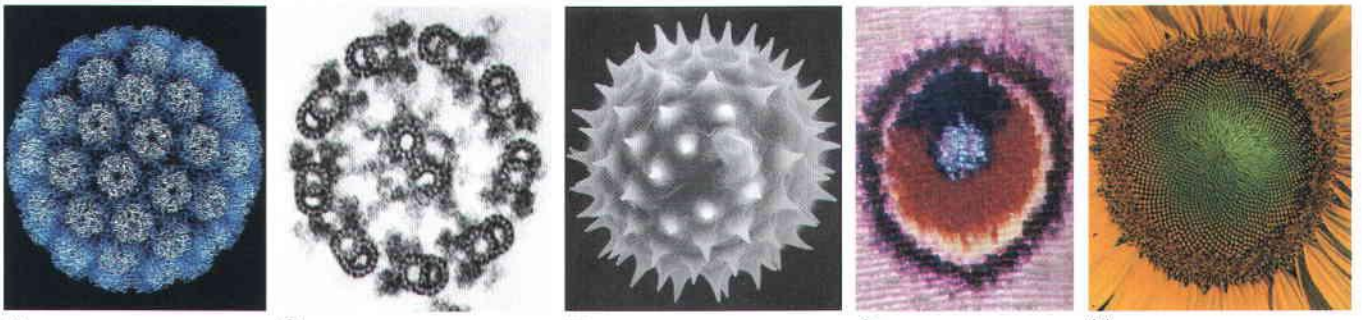


Figure 2–33 Order in biological structures. Well-defined, ornate, and beautiful spatial patterns can be found at every level of organization in living organisms. In order of increasing size: (A) protein molecules in the coat of a virus; (B) the regular array of microtubules seen in a cross section of a sperm tail; (C) surface contours of a pollen grain (a single cell); (D) close-up of the wing of a butterfly showing the pattern created by scales, each scale being the product of a single cell; (E) spiral array of seeds, made of millions of cells, in the head of a sunflower. (A, courtesy of R.A. Grant and J.M. Hogle; B, courtesy of Lewis Tilney; C, courtesy of Colin MacFarlane and Chris Jeffree; D and E, courtesy of Kjell B. Sandved.)

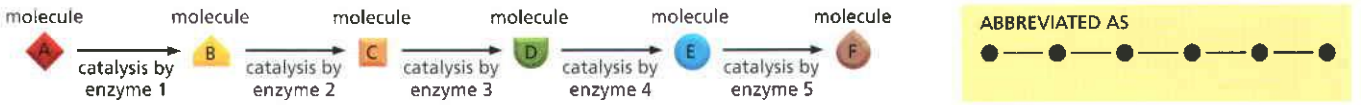


Figure 2–34 How a set of enzyme-catalyzed reactions generates a metabolic pathway. Each enzyme catalyzes a particular chemical reaction, leaving the enzyme unchanged. In this example, a set of enzymes acting in series converts molecule A to molecule F, forming a metabolic pathway.

Cell Metabolism Is Organized by Enzymes

The chemical reactions that a cell carries out would normally occur only at much higher temperatures than those existing inside cells. For this reason, each reaction requires a specific boost in chemical reactivity. This requirement is crucial, because it allows the cell to control each reaction. The control is exerted through the specialized proteins called *enzymes*, each of which accelerates, or *catalyzes*, just one of the many possible kinds of reactions that a particular molecule might undergo. Enzyme-catalyzed reactions are usually connected in series, so that the product of one reaction becomes the starting material, or *substrate*, for the next (**Figure 2–34**). These long linear reaction pathways are in turn linked to one another, forming a maze of interconnected reactions that enable the cell to survive, grow, and reproduce (**Figure 2–35**).

Two opposing streams of chemical reactions occur in cells: (1) the *catabolic* pathways break down foodstuffs into smaller molecules, thereby generating both a useful form of energy for the cell and some of the small molecules that the cell needs as building blocks, and (2) the *anabolic*, or *biosynthetic*, pathways use the energy harnessed by catabolism to drive the synthesis of the many other molecules that form the cell. Together these two sets of reactions constitute the **metabolism** of the cell (**Figure 2–36**).

Many of the details of cell metabolism form the traditional subject of *biochemistry* and need not concern us here. But the general principles by which cells obtain energy from their environment and use it to create order are central to cell biology. We begin with a discussion of why a constant input of energy is needed to sustain living organisms.

Biological Order Is Made Possible by the Release of Heat Energy from Cells

The universal tendency of things to become disordered is a fundamental law of physics—the *second law of thermodynamics*—which states that in the universe, or in any isolated system (a collection of matter that is completely isolated from the rest of the universe), the degree of disorder only increases. This law has such profound implications for all living things that we restate it in several ways.

For example, we can present the second law in terms of probability and state that systems will change spontaneously toward those arrangements that have the greatest probability. If we consider, for example, a box of 100 coins all lying heads up, a series of accidents that disturbs the box will tend to move the arrangement toward a mixture of 50 heads and 50 tails. The reason is simple: there is a huge number of possible arrangements of the individual coins in the mixture that can achieve the 50–50 result, but only one possible arrangement that keeps all of the coins oriented heads up. Because the 50–50 mixture is therefore the most probable, we say that it is more "disordered." For the same reason,

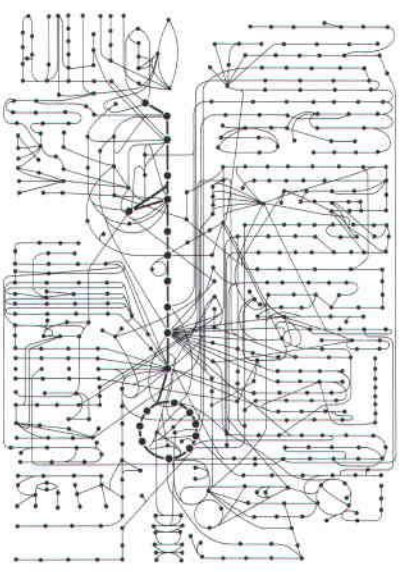


Figure 2–35 Some of the metabolic pathways and their interconnections in a typical cell. About 500 common metabolic reactions are shown diagrammatically, with each molecule in a metabolic pathway represented by a filled circle, as in the *yellow* box in Figure 2–34. The pathway that is highlighted in this diagram with larger circles and connecting lines is the central pathway of sugar metabolism, which will be discussed shortly.

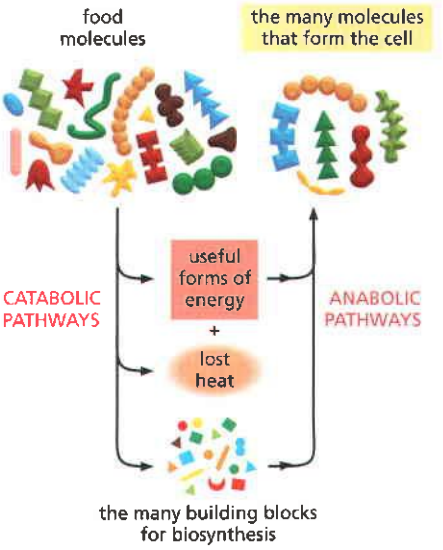


Figure 2–36 Schematic representation of the relationship between catabolic and anabolic pathways in metabolism. As suggested here, since a major portion of the energy stored in the chemical bonds of food molecules is dissipated as heat, the mass of food required by any organism that derives all of its energy from catabolism is much greater than the mass of the molecules that can be produced by anabolism.

it is a common experience that one's living space will become increasingly disordered without intentional effort: the movement toward disorder is a *spontaneous process*, requiring a periodic effort to reverse it (**Figure 2–37**).

The amount of disorder in a system can be quantified and expressed as the **entropy** of the system: the greater the disorder, the greater the entropy. Thus, another way to express the second law of thermodynamics is to say that systems will change spontaneously toward arrangements with greater entropy.

Living cells—by surviving, growing, and forming complex organisms—are generating order and thus might appear to defy the second law of thermodynamics. How is this possible? The answer is that a cell is not an isolated system: it takes in energy from its environment in the form of food, or as photons from the sun (or even, as in some chemosynthetic bacteria, from inorganic molecules alone), and it then uses this energy to generate order within itself. In the course of the chemical reactions that generate order, the cell converts part of the energy it uses into heat. The heat is discharged into the cell's environment and disorders it, so that the total entropy—that of the cell plus its surroundings—increases, as demanded by the laws of thermodynamics.

To understand the principles governing these energy conversions, think of a cell surrounded by a sea of matter representing the rest of the universe. As the cell lives and grows, it creates internal order. But it constantly releases heat energy as it synthesizes molecules and assembles them into cell structures. Heat is energy in its most disordered form—the random jostling of molecules. When the cell releases heat to the sea, it increases the intensity of molecular motions there (thermal motion)—thereby increasing the randomness, or disorder, of the sea. The second law of thermodynamics is satisfied because the increase in the amount of order inside the cell is more than compensated for by an even greater decrease in order (increase in entropy) in the surrounding sea of matter (**Figure 2–38**).

Where does the heat that the cell releases come from? Here we encounter another important law of thermodynamics. The *first law of thermodynamics* states that energy can be converted from one form to another, but that it cannot

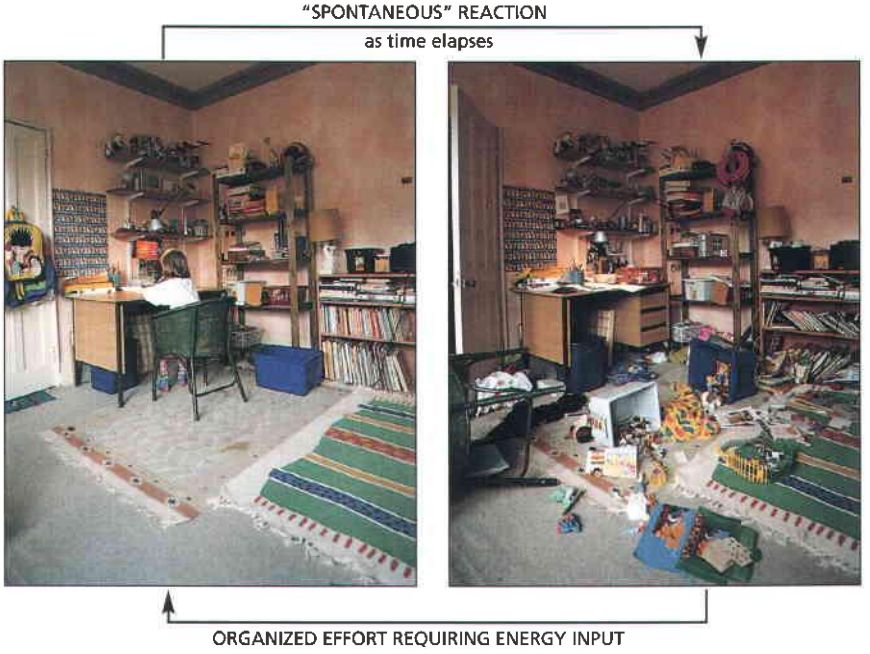


Figure 2–37 An everyday illustration of the spontaneous drive toward disorder. Reversing this tendency toward disorder requires an intentional effort and an input of energy: it is not spontaneous. In fact, from the second law of thermodynamics, we can be certain that the human intervention required will release enough heat to the environment to more than compensate for the reordering of the items in this room.

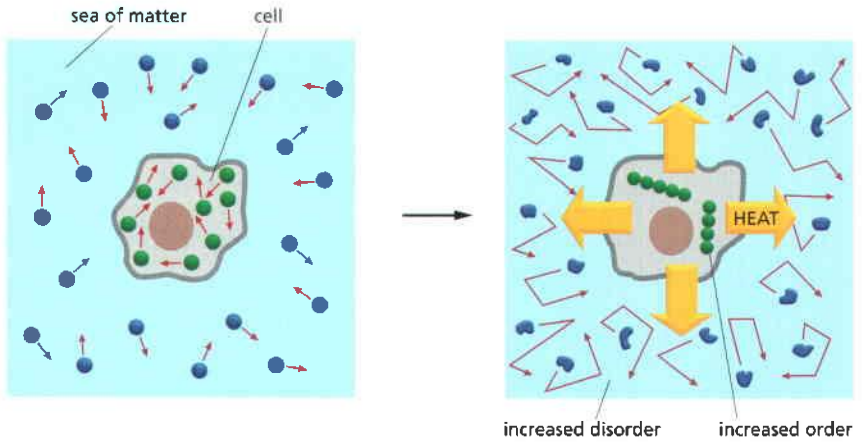


Figure 2–38 A simple thermodynamic analysis of a living cell. In the diagram on the left the molecules of both the cell and the rest of the universe (the sea of matter) are depicted in a relatively disordered state. In the diagram on the right the cell has taken in energy from food molecules and released heat by a reaction that orders the molecules the cell contains. Because the heat increases the disorder in the environment around the cell (depicted by the *jagged arrows* and distorted molecules, indicating the increased molecular motions caused by heat), the second law of thermodynamics—which states that the amount of disorder in the universe must always increase—is satisfied as the cell grows and divides. For a detailed discussion, see Panel 2–7 (pp. 118–119).

be created or destroyed. **Figure 2–39** illustrates some interconversions between different forms of energy. The amount of energy in different forms will change as a result of the chemical reactions inside the cell, but the first law tells us that the total amount of energy must always be the same. For example, an animal cell takes in foodstuffs and converts some of the energy present in the chemical bonds between the atoms of these food molecules (chemical bond energy) into the random thermal motion of molecules (heat energy). As described above, this conversion of chemical energy into heat energy is essential if the reactions that create order inside the cell are to cause the universe as a whole to become more disordered.

The cell cannot derive any benefit from the heat energy it releases unless the heat-generating reactions inside the cell are directly linked to the processes that generate molecular order. It is the tight *coupling* of heat production to an increase in order that distinguishes the metabolism of a cell from the wasteful burning of fuel in a fire. Later, we shall illustrate how this coupling occurs. For now, it is sufficient to recognize that a direct linkage of the "burning" of food molecules to the generation of biological order is required for cells to create and maintain an island of order in a universe tending toward chaos.

Photosynthetic Organisms Use Sunlight to Synthesize Organic Molecules

All animals live on energy stored in the chemical bonds of organic molecules made by other organisms, which they take in as food. The molecules in food also provide the atoms that animals need to construct new living matter. Some animals obtain their food by eating other animals. But at the bottom of the animal food chain are animals that eat plants. The plants, in turn, trap energy directly from sunlight. As a result, the sun is the ultimate source of the energy used by animal cells.

Solar energy enters the living world through **photosynthesis** in plants and photosynthetic bacteria. Photosynthesis converts the electromagnetic energy in sunlight into chemical bond energy in the cell. Plants obtain all the atoms they need from inorganic sources: carbon from atmospheric carbon dioxide, hydrogen and oxygen from water, nitrogen from ammonia and nitrates in the

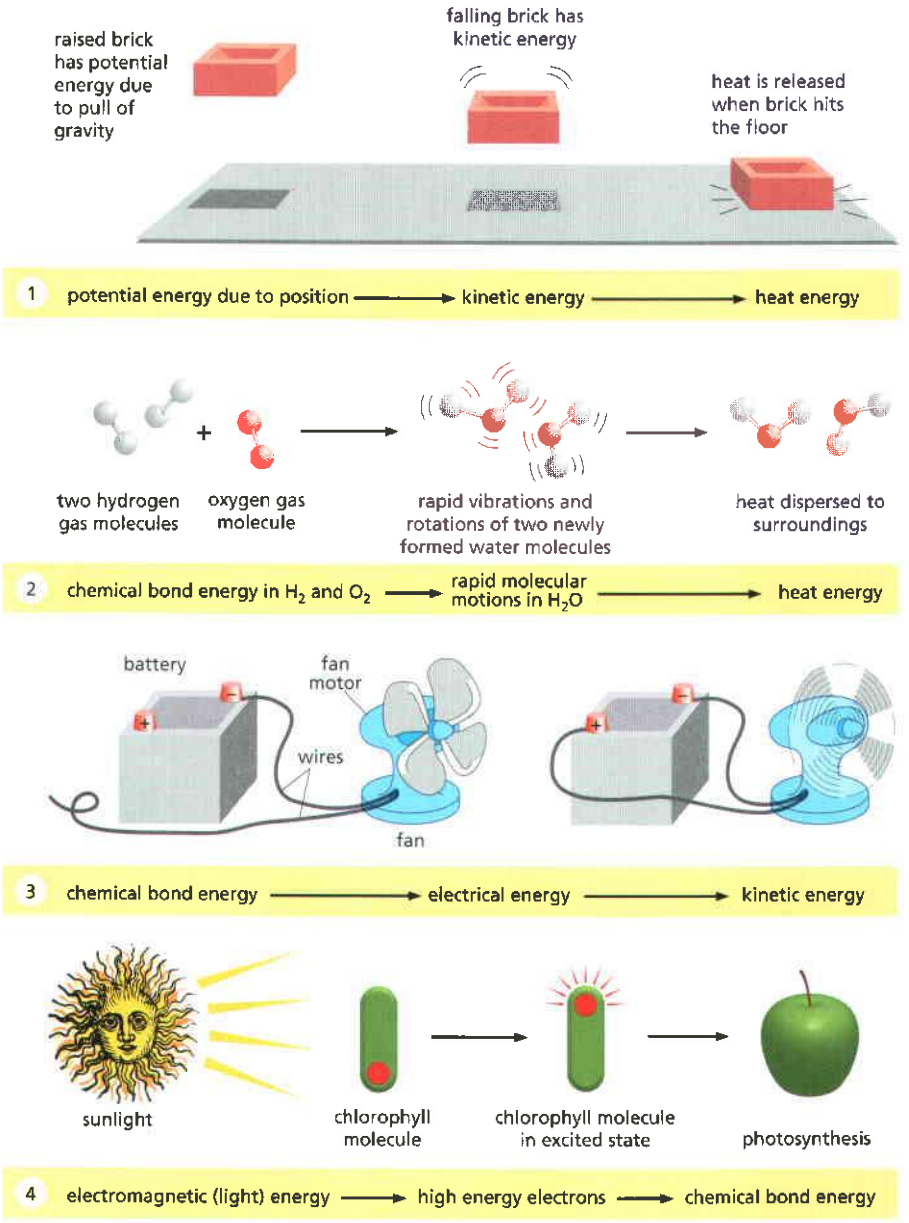


Figure 2–39 Some interconversions between different forms of energy. All energy forms are, in principle, interconvertible. In all these processes the total amount of energy is conserved. Thus, for example, from the height and weight of the brick in (1), we can predict exactly how much heat will be released when it hits the floor. In (2), note that the large amount of chemical bond energy released when water is formed is initially converted to very rapid thermal motions in the two new water molecules; but collisions with other molecules almost instantaneously spread this kinetic energy evenly throughout the surroundings (heat transfer), making the new molecules indistinguishable from all the rest.

soil, and other elements needed in smaller amounts from inorganic salts in the soil. They use the energy they derive from sunlight to build these atoms into sugars, amino acids, nucleotides, and fatty acids. These small molecules in turn are converted into the proteins, nucleic acids, polysaccharides, and lipids that form the plant. All of these substances serve as food molecules for animals, if the plants are later eaten.

The reactions of photosynthesis take place in two stages (**Figure 2–40**). In the first stage, energy from sunlight is captured and transiently stored as chemical bond energy in specialized small molecules that act as carriers of energy and reactive chemical groups. (We discuss these "activated carrier" molecules later.) Molecular oxygen (O_2 gas) derived from the splitting of water by light is released as a waste product of this first stage.

In the second stage, the molecules that serve as energy carriers are used to help drive a *carbon fixation* process in which sugars are manufactured from carbon dioxide gas (CO_2) and water (H_2O), thereby providing a useful source of stored chemical bond energy and materials—both for the plant itself and for any animals that eat it. We describe the elegant mechanisms that underlie these two stages of photosynthesis in Chapter 14.

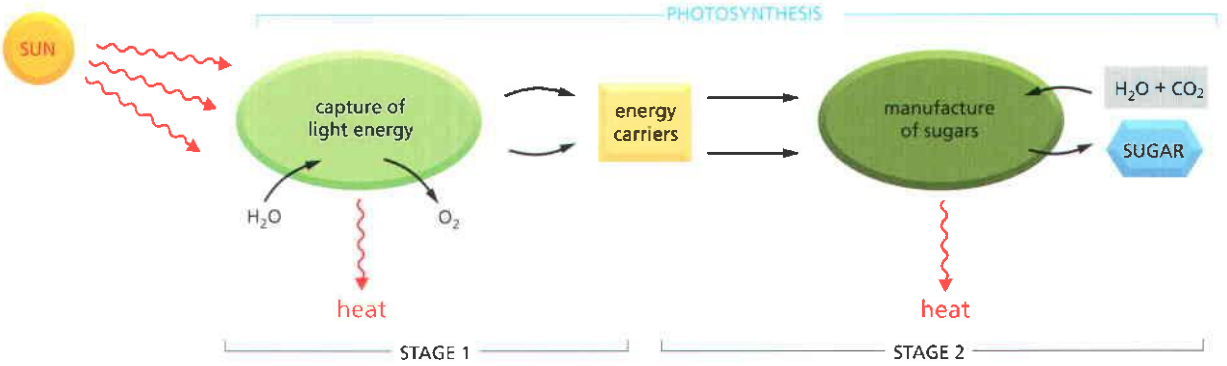


Figure 2–40 Photosynthesis. The two stages of photosynthesis. The energy carriers created in the first stage are two molecules that we discuss shortly—ATP and NADPH.

The net result of the entire process of photosynthesis, so far as the green plant is concerned, can be summarized simply in the equation

$$\text{light energy} + CO_2 + H_2O \rightarrow \text{sugars} + O_2 + \text{heat energy}$$

The sugars produced are then used both as a source of chemical bond energy and as a source of materials to make the many other small and large organic molecules that are essential to the plant cell.

Cells Obtain Energy by the Oxidation of Organic Molecules

All animal and plant cells are powered by energy stored in the chemical bonds of organic molecules, whether they are sugars that a plant has photosynthesized as food for itself or the mixture of large and small molecules that an animal has eaten. Organisms must extract this energy in usable form to live, grow, and reproduce. In both plants and animals, energy is extracted from food molecules by a process of gradual oxidation, or controlled burning.

The Earth's atmosphere contains a great deal of oxygen, and in the presence of oxygen the most energetically stable form of carbon is CO_2 and that of hydrogen is H_2O. A cell is therefore able to obtain energy from sugars or other organic molecules by allowing their carbon and hydrogen atoms to combine with oxygen to produce CO_2 and H_2O, respectively—a process called **respiration**.

Photosynthesis and respiration are complementary processes (**Figure 2–41**). This means that the transactions between plants and animals are not all one way. Plants, animals, and microorganisms have existed together on this planet for so long that many of them have become an essential part of the others' environments. The oxygen released by photosynthesis is consumed in the combustion of organic molecules by nearly all organisms. And some of the CO_2 molecules that are fixed today into organic molecules by photosynthesis in a green leaf were yesterday released into the atmosphere by the respiration of an animal—or by that of a fungus or bacterium decomposing dead organic matter. We therefore see that carbon utilization forms a huge cycle that involves the *biosphere* (all of the living organisms on Earth) as a whole, crossing boundaries

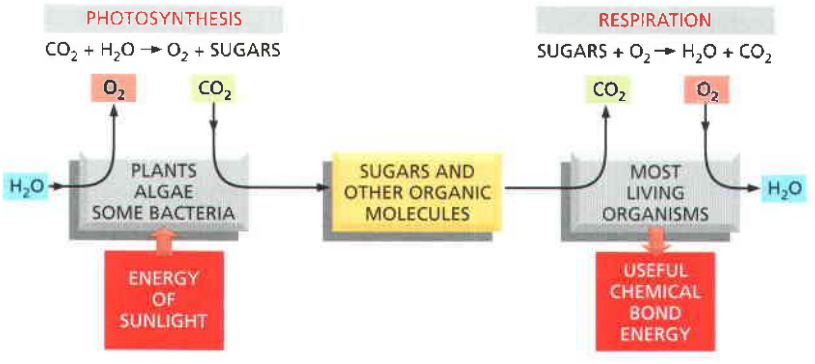


Figure 2–41 Photosynthesis and respiration as complementary processes in the living world. Photosynthesis uses the energy of sunlight to produce sugars and other organic molecules. These molecules in turn serve as food for other organisms. Many of these organisms carry out respiration, a process that uses O_2 to form CO_2 from the same carbon atoms that had been taken up as CO_2 and converted into sugars by photosynthesis. In the process, the organisms that respire obtain the chemical bond energy that they need to survive. The first cells on the Earth are thought to have been capable of neither photosynthesis nor respiration (discussed in Chapter 14). However, photosynthesis must have preceded respiration on the Earth, since there is strong evidence that billions of years of photosynthesis were required before O_2 had been released in sufficient quantity to create an atmosphere rich in this gas. (The Earth's atmosphere currently contains 20% O_2.)

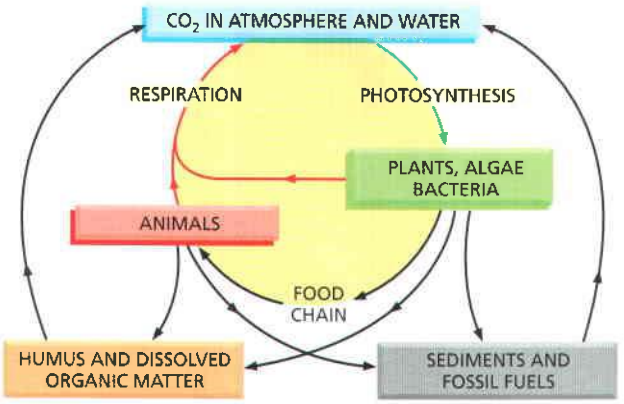


Figure 2–42 The carbon cycle. Individual carbon atoms are incorporated into organic molecules of the living world by the photosynthetic activity of bacteria and plants (including algae). They pass to animals, microorganisms, and organic material in soil and oceans in cyclic paths. CO_2 is restored to the atmosphere when organic molecules are oxidized by cells or burned by humans as fuels.

between individual organisms (**Figure 2–42**). Similarly, atoms of nitrogen, phosphorus, and sulfur move between the living and nonliving worlds in cycles that involve plants, animals, fungi, and bacteria.

Oxidation and Reduction Involve Electron Transfers

The cell does not oxidize organic molecules in one step, as occurs when organic material is burned in a fire. Through the use of enzyme catalysts, metabolism takes the molecules through a large number of reactions that only rarely involve the direct addition of oxygen. Before we consider some of these reactions and their purpose, we discuss what is meant by the process of oxidation.

Oxidation does not mean only the addition of oxygen atoms; rather, it applies more generally to any reaction in which electrons are transferred from one atom to another. Oxidation in this sense refers to the removal of electrons, and **reduction**—the converse of oxidation—means the addition of electrons. Thus, Fe^{2+} is oxidized if it loses an electron to become Fe^{3+}, and a chlorine atom is reduced if it gains an electron to become Cl^-. Since the number of electrons is conserved (no loss or gain) in a chemical reaction, oxidation and reduction always occur simultaneously: that is, if one molecule gains an electron in a reaction (reduction), a second molecule loses the electron (oxidation). When a sugar molecule is oxidized to CO_2 and H_2O, for example, the O_2 molecules involved in forming H_2O gain electrons and thus are said to have been reduced.

The terms "oxidation" and "reduction" apply even when there is only a partial shift of electrons between atoms linked by a covalent bond (**Figure 2–43**).

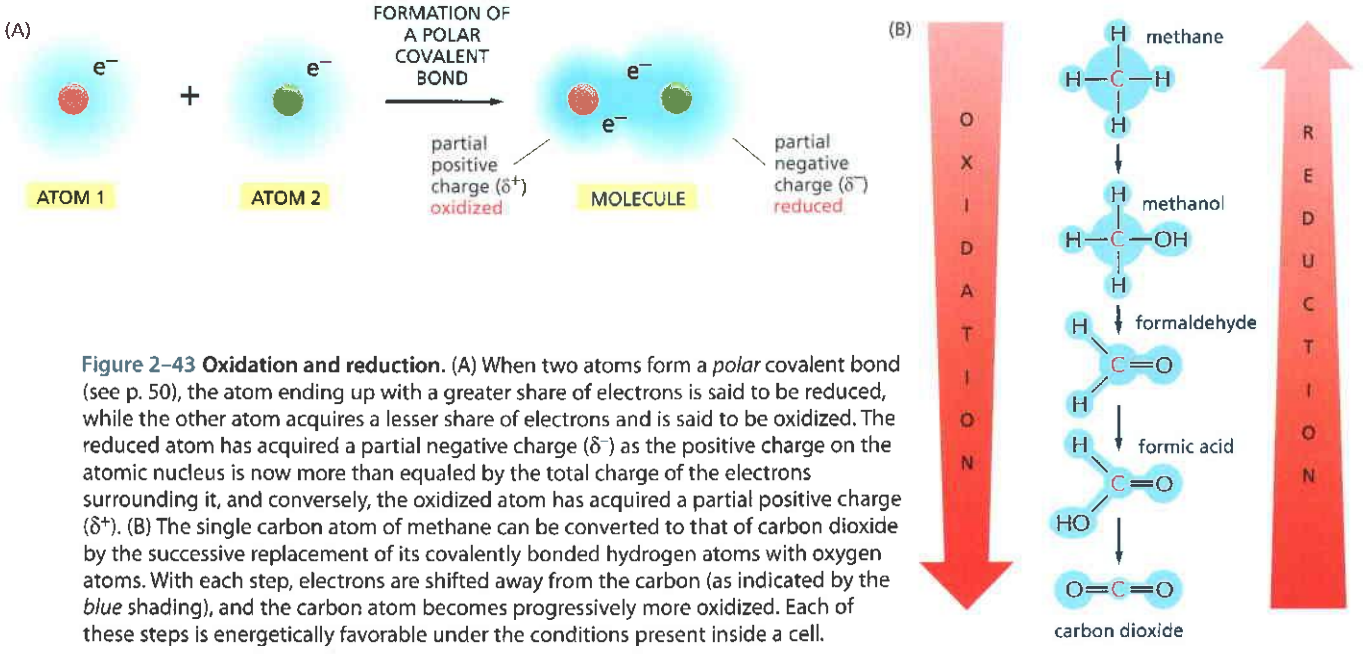


Figure 2–43 Oxidation and reduction. (A) When two atoms form a *polar* covalent bond (see p. 50), the atom ending up with a greater share of electrons is said to be reduced, while the other atom acquires a lesser share of electrons and is said to be oxidized. The reduced atom has acquired a partial negative charge (δ^-) as the positive charge on the atomic nucleus is now more than equaled by the total charge of the electrons surrounding it, and conversely, the oxidized atom has acquired a partial positive charge (δ^+). (B) The single carbon atom of methane can be converted to that of carbon dioxide by the successive replacement of its covalently bonded hydrogen atoms with oxygen atoms. With each step, electrons are shifted away from the carbon (as indicated by the *blue* shading), and the carbon atom becomes progressively more oxidized. Each of these steps is energetically favorable under the conditions present inside a cell.

When a carbon atom becomes covalently bonded to an atom with a strong affinity for electrons, such as oxygen, chlorine, or sulfur, for example, it gives up more than its equal share of electrons and forms a *polar* covalent bond: the positive charge of the carbon nucleus is now somewhat greater than the negative charge of its electrons, and the atom therefore acquires a partial positive charge and is said to be oxidized. Conversely, a carbon atom in a C–H linkage has slightly more than its share of electrons, and so it is said to be reduced (see Figure 2–43).

When a molecule in a cell picks up an electron (e^-), it often picks up a proton (H^+) at the same time (protons being freely available in water). The net effect in this case is to add a hydrogen atom to the molecule

$$A + e^- + H^+ \rightarrow AH$$

Even though a proton plus an electron is involved (instead of just an electron), such *hydrogenation* reactions are reductions, and the reverse, *dehydrogenation* reactions, are oxidations. It is especially easy to tell whether an organic molecule is being oxidized or reduced: reduction is occurring if its number of C–H bonds increases, whereas oxidation is occurring if its number of C–H bonds decreases (see Figure 2–43B).

Cells use enzymes to catalyze the oxidation of organic molecules in small steps, through a sequence of reactions that allows useful energy to be harvested. We now need to explain how enzymes work and some of the constraints under which they operate.

Enzymes Lower the Barriers That Block Chemical Reactions

Consider the reaction

$$\text{paper} + O_2 \rightarrow \text{smoke} + \text{ashes} + \text{heat} + CO_2 + H_2O$$

The paper burns readily, releasing to the atmosphere both energy as heat and water and carbon dioxide as gases, but the smoke and ashes never spontaneously retrieve these entities from the heated atmosphere and reconstitute themselves into paper. When the paper burns, its chemical energy is dissipated as heat—not lost from the universe, since energy can never be created or destroyed, but irretrievably dispersed in the chaotic random thermal motions of molecules. At the same time, the atoms and molecules of the paper become dispersed and disordered. In the language of thermodynamics, there has been a loss of *free energy*, that is, of energy that can be harnessed to do work or drive chemical reactions. This loss reflects a loss of orderliness in the way the energy and molecules were stored in the paper. We shall discuss free energy in more detail shortly, but the general principle is clear enough intuitively: chemical reactions proceed spontaneously only in the direction that leads to a loss of free energy; in other words, the spontaneous direction for any reaction is the direction that goes "downhill." A "downhill" reaction in this sense is often said to be *energetically favorable*.

Although the most energetically favorable form of carbon under ordinary conditions is CO_2, and that of hydrogen is H_2O, a living organism does not disappear in a puff of smoke, and the book in your hands does not burst into flames. This is because the molecules both in the living organism and in the book are in a relatively stable state, and they cannot be changed to a state of lower energy without an input of energy: in other words, a molecule requires **activation energy**—a kick over an energy barrier—before it can undergo a chemical reaction that leaves it in a more stable state (**Figure 2–44**). In the case of a burning book, the activation energy is provided by the heat of a lighted match. For the molecules in the watery solution inside a cell, the kick is delivered by an unusually energetic random collision with surrounding molecules—collisions that become more violent as the temperature is raised.

In a living cell, the kick over the energy barrier is greatly aided by a specialized class of proteins—the **enzymes**. Each enzyme binds tightly to one or more molecules, called **substrates**, and holds them in a way that greatly reduces the activation energy of a particular chemical reaction that the bound substrates can undergo. A substance that can lower the activation energy of a reaction is

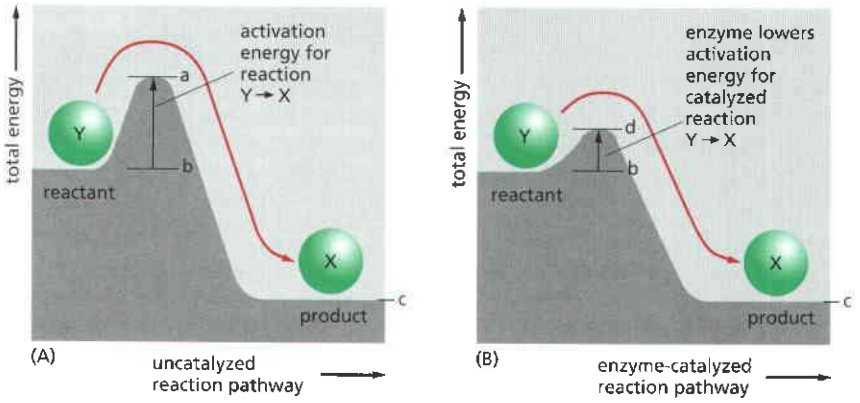


Figure 2–44 The important principle of activation energy. (A) Compound Y (a reactant) is in a relatively stable state, and energy is required to convert it to compound X (a product), even though X is at a lower overall energy level than Y. This conversion will not take place, therefore, unless compound Y can acquire enough activation energy *(energy a minus energy b)* from its surroundings to undergo the reaction that converts it into compound X. This energy may be provided by means of an unusually energetic collision with other molecules. For the reverse reaction, X → Y, the activation energy will be much larger *(energy a minus energy c);* this reaction will therefore occur much more rarely. Activation energies are always positive; note, however, that the total energy change for the energetically favorable reaction Y → X is *energy c minus energy b,* a negative number. (B) Energy barriers for specific reactions can be lowered by catalysts, as indicated by the line marked *d*. Enzymes are particularly effective catalysts because they greatly reduce the activation energy for the reactions they perform.

termed a **catalyst**; catalysts increase the rate of chemical reactions because they allow a much larger proportion of the random collisions with surrounding molecules to kick the substrates over the energy barrier, as illustrated in Figure 2–45. Enzymes are among the most effective catalysts known, capable of speeding up reactions by factors of 10^{14} or more. They thereby allow reactions that would not otherwise occur to proceed rapidly at normal temperatures.

Enzymes are also highly selective. Each enzyme usually catalyzes only one particular reaction: in other words, it selectively lowers the activation energy of only one of the several possible chemical reactions that its bound substrate molecules could undergo. In this way, enzymes direct each of the many different molecules in a cell along specific reaction pathways (Figure 2–46).

The success of living organisms is attributable to a cell's ability to make enzymes of many types, each with precisely specified properties. Each enzyme has a unique shape containing an *active site,* a pocket or groove in the enzyme into which only particular substrates will fit (Figure 2–47). Like all other catalysts, enzyme molecules themselves remain unchanged after participating in a reaction and therefore can function over and over again. In Chapter 3, we discuss further how enzymes work.

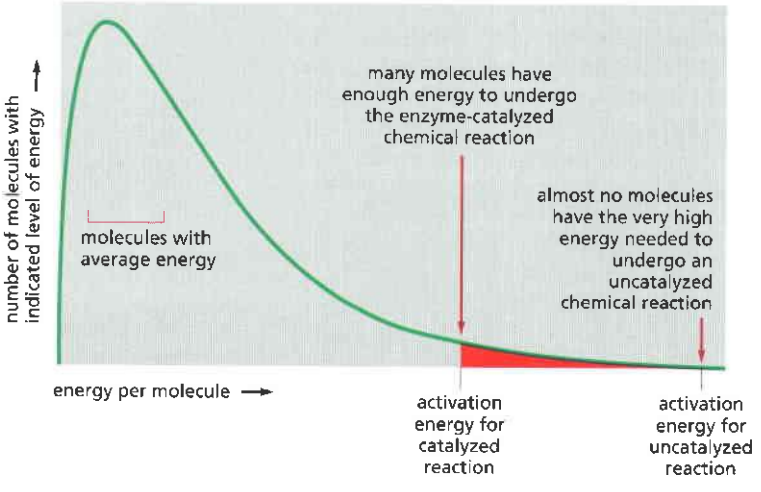


Figure 2–45 Lowering the activation energy greatly increases the probability of reaction. At any given instant, a population of identical substrate molecules will have a range of energies, distributed as shown on the graph. The varying energies come from collisions with surrounding molecules, which make the substrate molecules jiggle, vibrate, and spin. For a molecule to undergo a chemical reaction, the energy of the molecule must exceed the activation energy barrier for that reaction; for most biological reactions, this almost never happens without enzyme catalysis. Even with enzyme catalysis, the substrate molecules must experience a particularly energetic collision to react *(red shaded area).* Raising the temperature can also increase the number of molecules with sufficient energy to overcome the activation energy needed for a reaction; but in contrast to enzyme catalysis, this effect is nonselective, speeding up all reactions.

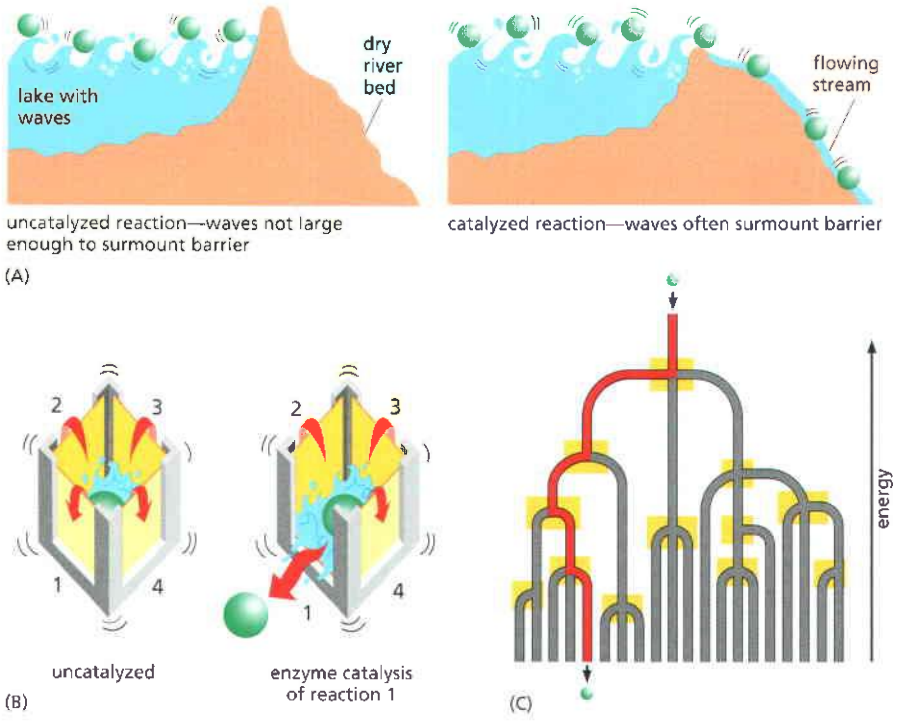


Figure 2–46 Floating ball analogies for enzyme catalysis. <TAAA> (A) A barrier dam is lowered to represent enzyme catalysis. The *green ball* represents a potential reactant (compound Y) that is bouncing up and down in energy level due to constant encounters with waves (an analogy for the thermal bombardment of the reactant molecule with the surrounding water molecules). When the barrier (activation energy) is lowered significantly, it allows the energetically favorable movement of the ball (the reactant) downhill. (B) The four walls of the box represent the activation energy barriers for four different chemical reactions that are all energetically favorable, in the sense that the products are at lower energy levels than the reactants. In the *left-hand* box, none of these reactions occurs because even the largest waves are not large enough to surmount any of the energy barriers. In the *right-hand* box, enzyme catalysis lowers the activation energy for reaction number 1 only; now the jostling of the waves allows passage of the reactant molecule over this energy barrier, inducing reaction 1. (C) A branching river with a set of barrier dams *(yellow boxes)* serves to illustrate how a series of enzyme-catalyzed reactions determines the exact reaction pathway followed by each molecule inside the cell.

How Enzymes Find Their Substrates: The Enormous Rapidity of Molecular Motions

An enzyme will often catalyze the reaction of thousands of substrate molecules every second. This means that it must be able to bind a new substrate molecule in a fraction of a millisecond. But both enzymes and their substrates are present in relatively small numbers in a cell. How do they find each other so fast? Rapid binding is possible because the motions caused by heat energy are enormously fast at the molecular level. These molecular motions can be classified broadly into three kinds: (1) the movement of a molecule from one place to another (*translational motion*), (2) the rapid back-and-forth movement of covalently linked atoms with respect to one another (vibrations), and (3) rotations. All of these motions help to bring the surfaces of interacting molecules together.

The rates of molecular motions can be measured by a variety of spectroscopic techniques. A large globular protein is constantly tumbling, rotating about its axis about a million times per second. Molecules are also in constant translational motion, which causes them to explore the space inside the cell very efficiently by wandering through it—a process called **diffusion**. In this way, every molecule in a cell collides with a huge number of other molecules each second. As the molecules in a liquid collide and bounce off one another, an individual molecule moves first one way and then another, its path constituting a *random walk* (**Figure 2–48**). In such a walk, the average net distance that each molecule travels (as the crow flies) from its starting point is proportional to the square root of the time involved: that is, if it takes a molecule 1 second on average to travel 1 μm, it takes 4 seconds to travel 2 μm, 100 seconds to travel 10 μm, and so on.

The inside of a cell is very crowded (**Figure 2–49**). Nevertheless, experiments in which fluorescent dyes and other labeled molecules are injected into cells

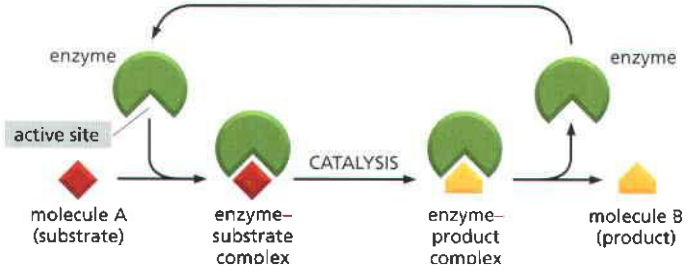


Figure 2–47 How enzymes work. Each enzyme has an active site to which one or more *substrate* molecules bind, forming an enzyme–substrate complex. A reaction occurs at the active site, producing an enzyme–product complex. The *product* is then released, allowing the enzyme to bind further substrate molecules.

show that small organic molecules diffuse through the watery gel of the cytosol nearly as rapidly as they do through water. A small organic molecule, for example, takes only about one-fifth of a second on average to diffuse a distance of 10 μm. Diffusion is therefore an efficient way for small molecules to move the limited distances in the cell (a typical animal cell is 15 μm in diameter).

Since enzymes move more slowly than substrates in cells, we can think of them as sitting still. The rate of encounter of each enzyme molecule with its substrate will depend on the concentration of the substrate molecule. For example, some abundant substrates are present at a concentration of 0.5 mM. Since pure water is 55.5 M, there is only about one such substrate molecule in the cell for every 10^5 water molecules. Nevertheless, the active site on an enzyme molecule that binds this substrate will be bombarded by about 500,000 random collisions with the substrate molecule per second. (For a substrate concentration tenfold lower, the number of collisions drops to 50,000 per second, and so on.) A random encounter between the surface of an enzyme and the matching surface of its substrate molecule often leads immediately to the formation of an enzyme–substrate complex that is ready to react. A reaction in which a covalent bond is broken or formed can now occur extremely rapidly. When one appreciates how quickly molecules move and react, the observed rates of enzymatic catalysis do not seem so amazing.

Once an enzyme and substrate have collided and snuggled together properly at the active site, they form multiple weak bonds with each other that persist until random thermal motion causes the molecules to dissociate again. In general, the stronger the binding of the enzyme and substrate, the slower their rate of dissociation. However, when two colliding molecules have poorly matching surfaces, they form few noncovalent bonds and their total energy is negligible compared with that of thermal motion. In this case the two molecules dissociate as rapidly as they come together, preventing incorrect and unwanted associations between mismatched molecules, such as between an enzyme and the wrong substrate.

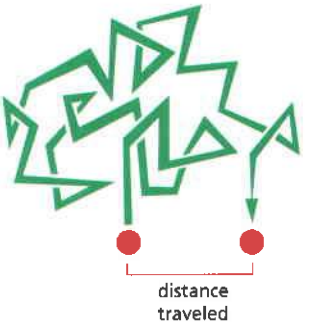


Figure 2–48 A random walk. <GGTA> Molecules in solution move in a random fashion as a result of the continual buffeting they receive in collisions with other molecules. This movement allows small molecules to diffuse rapidly from one part of the cell to another, as described in the text.

The Free-Energy Change for a Reaction Determines Whether It Can Occur

We must now digress briefly to introduce some fundamental chemistry. Cells are chemical systems that must obey all chemical and physical laws. Although enzymes speed up reactions, they cannot by themselves force energetically unfavorable reactions to occur. In terms of a water analogy, enzymes by themselves cannot make water run uphill. Cells, however, must do just that in order to grow and divide: they must build highly ordered and energy-rich molecules from small and simple ones. We shall see that this is done through enzymes that directly *couple* energetically favorable reactions, which release energy and produce heat, to energetically unfavorable reactions, which produce biological order.

Before examining how such coupling is achieved, we must consider more carefully the term "energetically favorable." According to the second law of thermodynamics, a chemical reaction can proceed spontaneously only if it results in a net increase in the disorder of the universe (see Figure 2–38). The criterion for an increase in disorder of the universe can be expressed most conveniently in terms of a quantity called the **free energy**, ***G***, of a system. The value of G is of interest only when a system undergoes a *change*, and the change in G, denoted ΔG (delta G), is critical. Suppose that the system being considered is a collection of molecules. As explained in Panel 2–7 (pp. 118–119), free energy has been defined such that ΔG directly measures the amount of disorder created in the universe when a reaction takes place that involves these molecules. *Energetically favorable reactions*, by definition, are those that decrease free energy; in other words, they have a *negative* ΔG and disorder the universe (**Figure 2–50**).

An example of an energetically favorable reaction on a macroscopic scale is the "reaction" by which a compressed spring relaxes to an expanded state, releasing its stored elastic energy as heat to its surroundings; an example on a microscopic scale is salt dissolving in water. Conversely, *energetically unfavorable reactions*, with a *positive* ΔG—such as the joining of two amino acids to

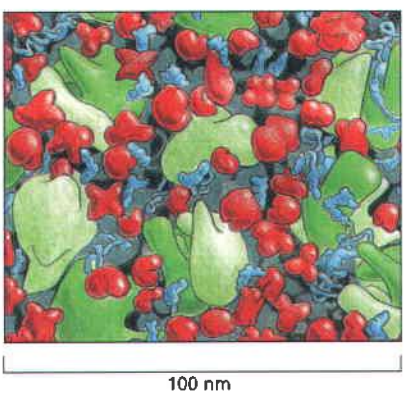


Figure 2–49 The structure of the cytoplasm. The drawing is approximately to scale and emphasizes the crowding in the cytoplasm. Only the macromolecules are shown: RNAs are shown in *blue*, ribosomes in *green*, and proteins in *red*. Enzymes and other macromolecules diffuse relatively slowly in the cytoplasm, in part because they interact with many other macromolecules; small molecules, by contrast, diffuse nearly as rapidly as they do in water. (Adapted from D.S. Goodsell, *Trends Biochem. Sci.* 16:203–206, 1991. With permission from Elsevier.)

form a peptide bond—by themselves create order in the universe. Therefore, these reactions can take place only if they are coupled to a second reaction with a negative ΔG so large that the ΔG of the entire process is negative (**Figure 2–51**).

The Concentration of Reactants Influences the Free-Energy Change and a Reaction's Direction

As we have just described, a reaction $Y \rightleftharpoons X$ will go in the direction $Y \rightarrow X$ when the associated free-energy change, ΔG, is negative, just as a tensed spring left to itself will relax and lose its stored energy to its surroundings as heat. For a chemical reaction, however, ΔG depends not only on the energy stored in each individual molecule, but also on the concentrations of the molecules in the reaction mixture. Remember that ΔG reflects the degree to which a reaction creates a more disordered—in other words, a more probable—state of the universe. Recalling our coin analogy, it is very likely that a coin will flip from a head to a tail orientation if a jiggling box contains 90 heads and 10 tails, but this is a less probable event if the box has 10 heads and 90 tails.

The same is true for a chemical reaction. For a reversible reaction $Y \rightleftharpoons X$, a large excess of Y over X will tend to drive the reaction in the direction $Y \rightarrow X$; that is, there will be a tendency for there to be more molecules making the transition $Y \rightarrow X$ than there are molecules making the transition $X \rightarrow Y$. If the ratio of Y to X increases, the ΔG becomes more negative for the transition $Y \rightarrow X$ (and more positive for the transition $X \rightarrow Y$).

How much of a concentration difference is needed to compensate for a given decrease in chemical bond energy (and accompanying heat release)? The answer is not intuitively obvious, but it can be determined from a thermodynamic analysis that makes it possible to separate the concentration-dependent and the concentration-independent parts of the free-energy change. The ΔG for a given reaction can thereby be written as the sum of two parts: the first, called the *standard free-energy change*, $\Delta G°$, depends on the intrinsic characters of the reacting molecules; the second depends on their concentrations. For the simple reaction $Y \rightarrow X$ at 37°C,

$$\Delta G = \Delta G° + 0.616 \ln \frac{[X]}{[Y]} = \Delta G° + 1.42 \log \frac{[X]}{[Y]}$$

where ΔG is in kilocalories per mole, [Y] and [X] denote the concentrations of Y and X, ln is the natural logarithm, and the constant 0.616 is equal to RT: the product of the gas constant, R, and the absolute temperature, T.

Note that ΔG equals the value of $\Delta G°$ when the molar concentrations of Y and X are equal ($\log 1 = 0$). As expected, ΔG becomes more negative as the ratio of X to Y decreases (the log of a number < 1 is negative).

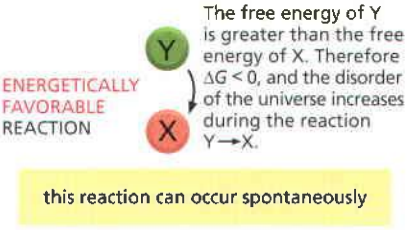
Inspection of the above equation reveals that the ΔG equals the value of $\Delta G°$ when the concentrations of Y and X are equal. But as the favorable reaction $Y \rightarrow X$ proceeds, the concentration of the product X increases and the concentration of the substrate Y decreases. This change in relative concentrations will cause [X]/[Y] to become increasingly large, making the initially favorable ΔG less and less negative. Eventually, when $\Delta G = 0$, a chemical **equilibrium** will be attained; here the concentration effect just balances the push given to the reaction by $\Delta G°$, and the ratio of substrate to product reaches a constant value (**Figure 2–52**).

How far will a reaction proceed before it stops at equilibrium? To address this question, we need to introduce the **equilibrium constant**, K. The value of K is different for different reactions, and it reflects the ratio of product to substrate at equilibrium. For the reaction $Y \rightarrow X$:

$$K = \frac{[X]}{[Y]}$$

The equation that connects ΔG and the ratio [X]/[Y] allows us to connect $\Delta G°$ directly to K. Since $\Delta G = 0$ at equilibrium, the concentrations of Y and X at this point are such that:

$$\Delta G° = -1.42 \log \frac{[X]}{[Y]} \quad \text{or,} \quad \Delta G° = -1.42 \log K$$



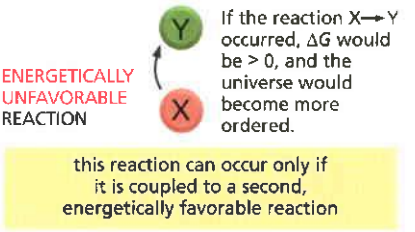


Figure 2–50 The distinction between energetically favorable and energetically unfavorable reactions.

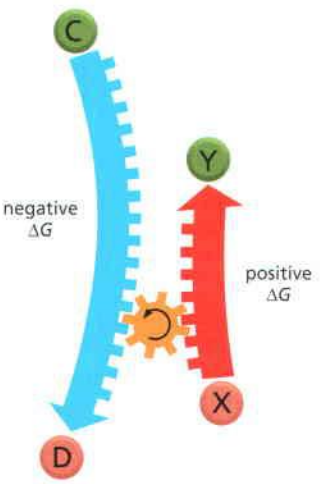


Figure 2–51 How reaction coupling is used to drive energetically unfavorable reactions.

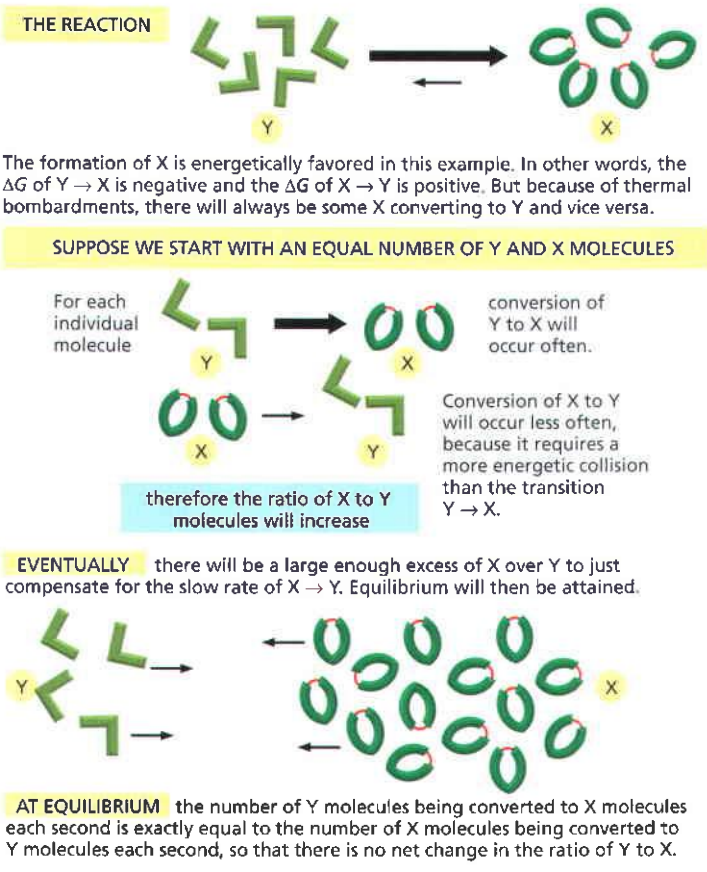


Figure 2–52 Chemical equilibrium. When a reaction reaches equilibrium, the forward and backward fluxes of reacting molecules are equal and opposite.

Using the last equation, we can see how the equilibrium ratio of X to Y (expressed as an equilibrium constant, K) depends on the intrinsic character of the molecules, as expressed in the value of $\Delta G°$ (Table 2–4). Note that for every 1.4 kcal/mole (5.9 kJ/mole) difference in free energy at 37°C, the equilibrium constant changes by a factor of 10.

When an enzyme (or any catalyst) lowers the activation energy for the reaction Y → X, it also lowers the activation energy for the reaction X → Y by exactly the same amount (see Figure 2–44). The forward and backward reactions will therefore be accelerated by the same factor by an enzyme, and the equilibrium point for the reaction (and $\Delta G°$) is unchanged (Figure 2–53).

Table 2–4 Relationship Between the Standard Free-Energy Change, $\Delta G°$, and the Equilibrium Constant

EQUILIBRIUM CONSTANT $\frac{[X]}{[Y]} = K$	FREE ENERGY OF X MINUS FREE ENERGY OF Y kcal/mole (kJ/mole)
10^5	−7.1 (−29.7)
10^4	−5.7 (−23.8)
10^3	−4.3 (−18.0)
10^2	−2.8 (−11.7)
10^1	−1.4 (−5.9)
1	0 (0)
10	1.4 (5.9)
10^{-2}	2.8 (11.7)
10^{-3}	4.3 (18.0)
10^{-4}	5.7 (23.8)
10^{-5}	7.1 (29.7)

Values of the equilibrium constant were calculated for the simple chemical reaction Y ⇌ X using the equation given in the text.

The $\Delta G°$ given here is in kilocalories per mole at 37°C, with kilojoules per mole in parentheses (1 kilocalorie is equal to 4.184 kilojoules). As explained in the text, $\Delta G°$ represents the free-energy difference under standard conditions (where all components are present at a concentration of 1.0 mole/liter).

From this table, we see that if there is a favorable standard free-energy change ($\Delta G°$) of −4.3 kcal/mole (−18.0 kJ/mole) for the transition Y → X, there will be 1000 times more molecules in state X than in state Y at equilibrium (K = 1000).

For Sequential Reactions, $\Delta G°$ Values Are Additive

We can predict quantitatively the course of most reactions. A large body of thermodynamic data has been collected that makes it possible to calculate the standard change in free energy, $\Delta G°$, for most of the important metabolic reactions of the cell. The overall free-energy change for a metabolic pathway is then simply the sum of the free-energy changes in each of its component steps. Consider, for example, two sequential reactions

$$X \rightarrow Y \quad \text{and} \quad Y \rightarrow Z$$

whose $\Delta G°$ values are +5 and −13 kcal/mole, respectively. (Recall that a mole is 6×10^{23} molecules of a substance.) If these two reactions occur sequentially, the $\Delta G°$ for the coupled reaction will be −8 kcal/mole. Thus, the unfavorable reaction X → Y, which will not occur spontaneously, can be driven by the favorable reaction Y → Z, provided that this second reaction follows the first.

Cells can therefore cause the energetically unfavorable transition, X → Y, to occur if an enzyme catalyzing the X → Y reaction is supplemented by a second enzyme that catalyzes the energetically *favorable* reaction, Y → Z. In effect, the reaction Y → Z will then act as a "siphon" to drive the conversion of all of molecule X to molecule Y, and thence to molecule Z (Figure 2–54). For example,

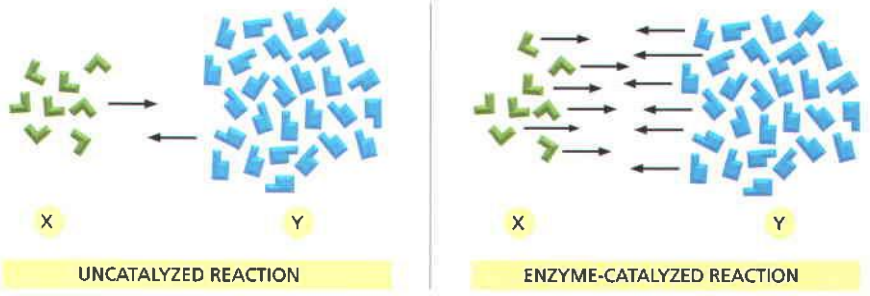


Figure 2–53 Enzymes cannot change the equilibrium point for reactions. Enzymes, like all catalysts, speed up the forward and backward rates of a reaction by the same factor. Therefore, for both the catalyzed and the uncatalyzed reactions shown here, the number of molecules undergoing the transition $X \rightarrow Y$ is equal to the number of molecules undergoing the transition $Y \rightarrow X$ when the ratio of Y molecules to X molecules is 3.5 to 1. In other words, the two reactions reach equilibrium at exactly the same point.

several of the reactions in the long pathway that converts sugars into CO_2 and H_2O would be energetically unfavorable if considered on their own. But the pathway nevertheless proceeds because the total $\Delta G°$ for the series of sequential reactions has a large negative value.

But forming a sequential pathway is not adequate for many purposes. Often the desired pathway is simply $X \rightarrow Y$, without further conversion of Y to some other product. Fortunately, there are other more general ways of using enzymes to couple reactions together. How these work is the topic we discuss next.

Activated Carrier Molecules Are Essential for Biosynthesis

The energy released by the oxidation of food molecules must be stored temporarily before it can be channeled into the construction of the many other molecules needed by the cell. In most cases, the energy is stored as chemical bond energy in a small set of activated "carrier molecules," which contain one or more energy-rich covalent bonds. These molecules diffuse rapidly throughout the cell and thereby carry their bond energy from sites of energy generation to the sites where energy is used for biosynthesis and other cell activities (**Figure 2–55**).

The **activated carriers** store energy in an easily exchangeable form, either as a readily transferable chemical group or as high-energy electrons, and they can serve a dual role as a source of both energy and chemical groups in biosynthetic reactions. For historical reasons, these molecules are also sometimes referred to as *coenzymes*. The most important of the activated carrier molecules are ATP and two molecules that are closely related to each other, NADH and NADPH—as we discuss in detail shortly. We shall see that cells use activated carrier molecules like money to pay for reactions that otherwise could not take place.

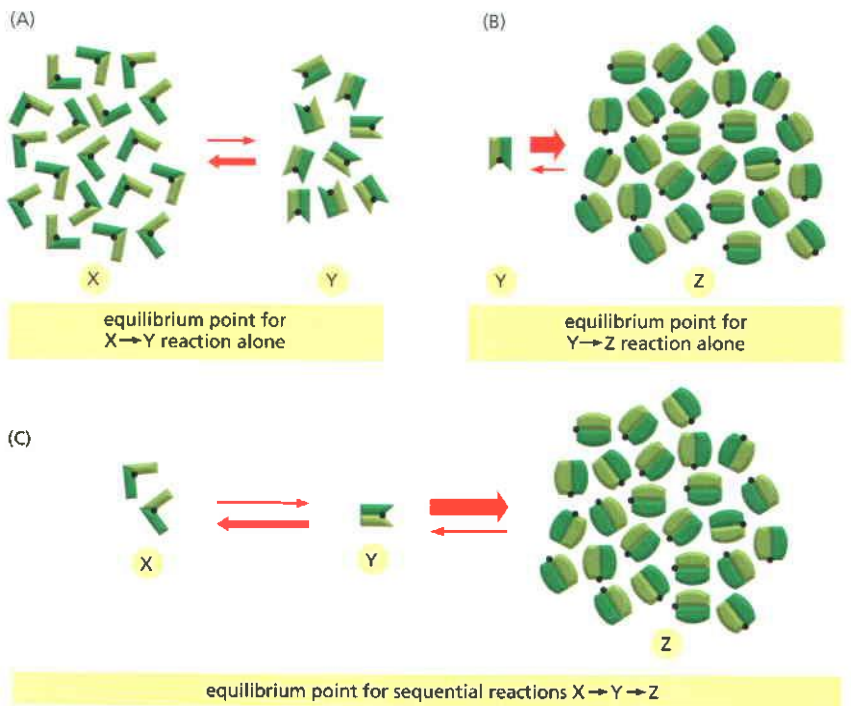


Figure 2–54 How an energetically unfavorable reaction can be driven by a second, following reaction. (A) At equilibrium, there are twice as many X molecules as Y molecules, because X is of lower energy than Y. (B) At equilibrium, there are 25 times more Z molecules than Y molecules, because Z is of much lower energy than Y. (C) If the reactions in (A) and (B) are coupled, nearly all of the X molecules will be converted to Z molecules, as shown.

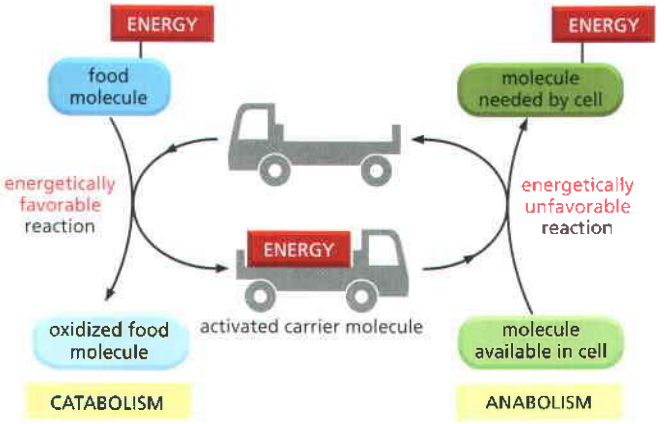


Figure 2–55 Energy transfer and the role of activated carriers in metabolism. By serving as energy shuttles, activated carrier molecules perform their function as go-betweens that link the breakdown of food molecules and the release of energy *(catabolism)* to the energy-requiring biosynthesis of small and large organic molecules *(anabolism)*.

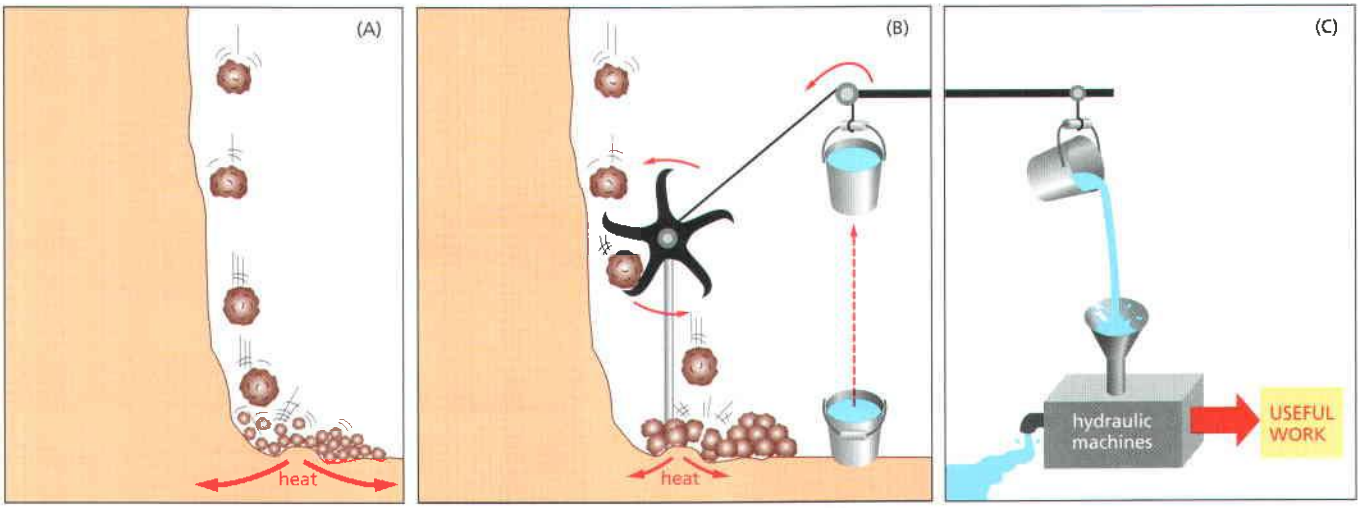
The Formation of an Activated Carrier Is Coupled to an Energetically Favorable Reaction

When a fuel molecule such as glucose is oxidized in a cell, enzyme-catalyzed reactions ensure that a large part of the free energy that is released by oxidation is captured in a chemically useful form, rather than being released as heat. This is achieved by means of a **coupled reaction**, in which an energetically favorable reaction drives an energetically unfavorable one that produces an activated carrier molecule or some other useful energy store. Coupling mechanisms require enzymes and are fundamental to all the energy transactions of the cell.

The nature of a coupled reaction is illustrated by a mechanical analogy in Figure 2–56, in which an energetically favorable chemical reaction is represented by rocks falling from a cliff. The energy of falling rocks would normally be entirely wasted in the form of heat generated by friction when the rocks hit the ground (see the falling brick diagram in Figure 2–39). By careful design, however, part of this energy could be used instead to drive a paddle wheel that lifts a bucket of water (Figure 2–56B). Because the rocks can now reach the ground only after moving the paddle wheel, we say that the energetically favorable reaction of rock falling has been directly *coupled* to the energetically unfavorable reaction of lifting the bucket of water. Note that because part of the energy is used to do work in (B), the rocks hit the ground with less velocity than in (A), and correspondingly less energy is dissipated as heat.

Similar processes occur in cells, where enzymes play the role of the paddle wheel in our analogy. By mechanisms that will be discussed later in this chapter, they couple an energetically favorable reaction, such as the oxidation of foodstuffs, to an energetically unfavorable reaction, such as the generation of

Figure 2–56 A mechanical model illustrating the principle of coupled chemical reactions. The spontaneous reaction shown in (A) could serve as an analogy for the direct oxidation of glucose to CO_2 and H_2O, which produces heat only. In (B) the same reaction is coupled to a second reaction; this second reaction is analogous to the synthesis of activated carrier molecules. The energy produced in (B) is in a more useful form than in (A) and can be used to drive a variety of otherwise energetically unfavorable reactions (C).



kinetic energy of falling rocks is transformed into heat energy only

part of the kinetic energy is used to lift a bucket of water, and a correspondingly smaller amount is transformed into heat

the potential kinetic energy stored in the raised bucket of water can be used to drive hydraulic machines that carry out a variety of useful tasks

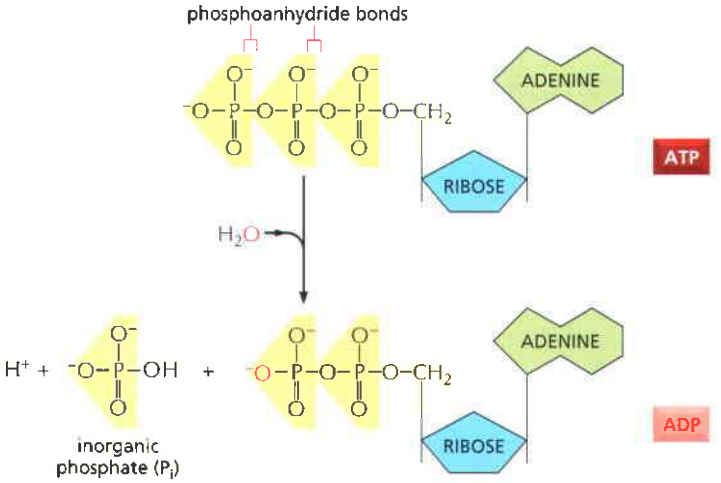


Figure 2–57 The hydrolysis of ATP to ADP and inorganic phosphate. The two outermost phosphates in ATP are held to the rest of the molecule by high-energy phosphoanhydride bonds and are readily transferred. As indicated, water can be added to ATP to form ADP and inorganic phosphate (P_i). This hydrolysis of the terminal phosphate of ATP yields between 11 and 13 kcal/mole of usable energy, depending on the intracellular conditions. The large negative ΔG of this reaction arises from several factors. Release of the terminal phosphate group removes an unfavorable repulsion between adjacent negative charges; in addition, the inorganic phosphate ion (P_i) released is stabilized by resonance and by favorable hydrogen-bond formation with water.

an activated carrier molecule. As a result, the amount of heat released by the oxidation reaction is reduced by exactly the amount of energy that is stored in the energy-rich covalent bonds of the activated carrier molecule. The activated carrier molecule in turn picks up a packet of energy of a size sufficient to power a chemical reaction elsewhere in the cell.

ATP Is the Most Widely Used Activated Carrier Molecule

The most important and versatile of the activated carriers in cells is **ATP** (adenosine triphosphate). Just as the energy stored in the raised bucket of water in Figure 2–56B can drive a wide variety of hydraulic machines, ATP is a convenient and versatile store, or currency, of energy used to drive a variety of chemical reactions in cells. ATP is synthesized in an energetically unfavorable phosphorylation reaction in which a phosphate group is added to **ADP** (adenosine diphosphate). When required, ATP gives up its energy packet through its energetically favorable hydrolysis to ADP and inorganic phosphate (**Figure 2–57**). The regenerated ADP is then available to be used for another round of the phosphorylation reaction that forms ATP.

The energetically favorable reaction of ATP hydrolysis is coupled to many otherwise unfavorable reactions through which other molecules are synthesized. We shall encounter several of these reactions later in this chapter. Many of them involve the transfer of the terminal phosphate in ATP to another molecule, as illustrated by the phosphorylation reaction in **Figure 2–58**.

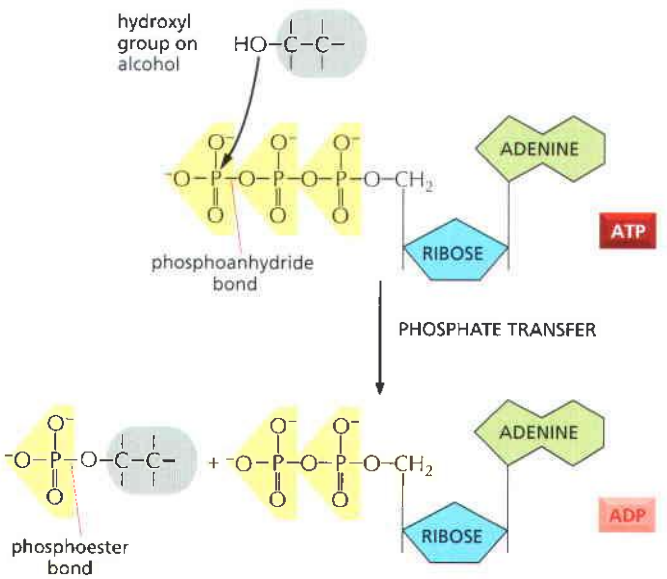


Figure 2–58 An example of a phosphate transfer reaction. Because an energy-rich phosphoanhydride bond in ATP is converted to a phosphoester bond, this reaction is energetically favorable, having a large negative ΔG. Reactions of this type are involved in the synthesis of phospholipids and in the initial steps of reactions that catabolize sugars.

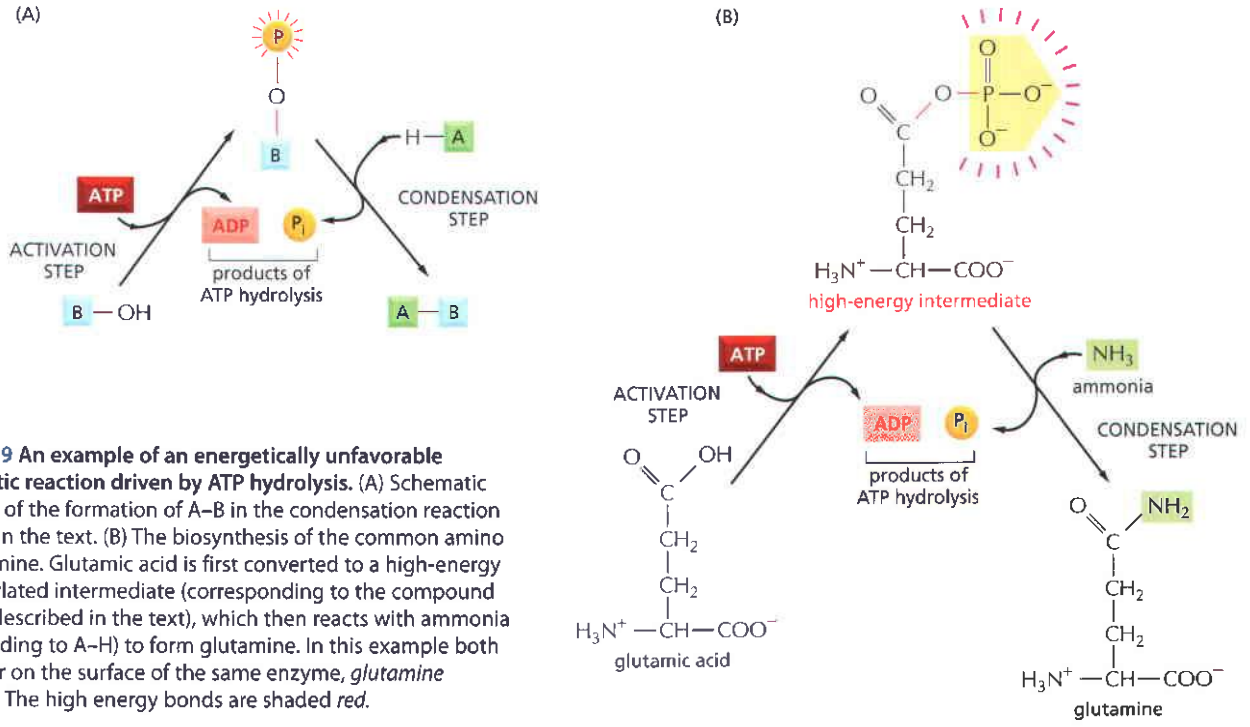


Figure 2–59 An example of an energetically unfavorable biosynthetic reaction driven by ATP hydrolysis. (A) Schematic illustration of the formation of A–B in the condensation reaction described in the text. (B) The biosynthesis of the common amino acid glutamine. Glutamic acid is first converted to a high-energy phosphorylated intermediate (corresponding to the compound B–O–PO_3 described in the text), which then reacts with ammonia (corresponding to A–H) to form glutamine. In this example both steps occur on the surface of the same enzyme, *glutamine synthetase*. The high energy bonds are shaded *red*.

ATP is the most abundant activated carrier in cells. As one example, it supplies energy for many of the pumps that transport substances into and out of the cell (discussed in Chapter 11). It also powers the molecular motors that enable muscle cells to contract and nerve cells to transport materials from one end of their long axons to another (discussed in Chapter 16).

Energy Stored in ATP Is Often Harnessed to Join Two Molecules Together

We have previously discussed one way in which an energetically favorable reaction can be coupled to an energetically unfavorable reaction, X → Y, so as to enable it to occur. In that scheme a second enzyme catalyzes the energetically favorable reaction Y → Z, pulling all of the X to Y in the process (see Figure 2–54). But when the required product is Y and not Z, this mechanism is not useful.

A typical biosynthetic reaction is one in which two molecules, A and B, are joined together to produce A–B in the energetically unfavorable condensation reaction

$$A\text{–}H + B\text{–}OH \rightarrow A\text{–}B + H_2O$$

There is an indirect pathway that allows A–H and B–OH to form A–B, in which a coupling to ATP hydrolysis makes the reaction go. Here energy from ATP hydrolysis is first used to convert B–OH to a higher-energy intermediate compound, which then reacts directly with A–H to give A–B. The simplest possible mechanism involves the transfer of a phosphate from ATP to B–OH to make B–OPO_3, in which case the reaction pathway contains only two steps:

1. $B\text{–}OH + ATP \rightarrow B\text{–}O\text{–}PO_3 + ADP$
2. $A\text{–}H + B\text{–}O\text{–}PO_3 \rightarrow A\text{–}B + P_i$

Net result: $B\text{–}OH + ATP + A\text{–}H \rightarrow A\text{–}B + ADP + P_i$

The condensation reaction, which by itself is energetically unfavorable, is forced to occur by being directly coupled to ATP hydrolysis in an enzyme-catalyzed reaction pathway (**Figure 2–59**A).

A biosynthetic reaction of exactly this type synthesizes the amino acid glutamine (Figure 2–59B). We will see shortly that similar (but more complex) mechanisms are also used to produce nearly all of the large molecules of the cell.

NADH and NADPH Are Important Electron Carriers

Other important activated carrier molecules participate in oxidation–reduction reactions and are commonly part of coupled reactions in cells. These activated carriers are specialized to carry high-energy electrons and hydrogen atoms. The most important of these electron carriers are **NAD^+** (nicotinamide adenine dinucleotide) and the closely related molecule **$NADP^+$** (nicotinamide adenine dinucleotide phosphate). Later, we examine some of the reactions in which they participate. NAD^+ and $NADP^+$ each pick up a "packet of energy" corresponding to two high-energy electrons plus a proton (H^+)—being converted to **NADH** (*reduced* nicotinamide adenine dinucleotide) and **NADPH** (*reduced* nicotinamide adenine dinucleotide phosphate), respectively. These molecules can therefore also be regarded as carriers of hydride ions (the H^+ plus two electrons, or H^-).

Like ATP, NADPH is an activated carrier that participates in many important biosynthetic reactions that would otherwise be energetically unfavorable. The NADPH is produced according to the general scheme shown in Figure 2–60A. During a special set of energy-yielding catabolic reactions, a hydrogen atom plus two electrons are removed from the substrate molecule and added to the nicotinamide ring of $NADP^+$ to form NADPH, with a proton (H^+) being released into solution. This is a typical oxidation–reduction reaction; the substrate is oxidized and $NADP^+$ is reduced. The structures of $NADP^+$ and NADPH are shown in Figure 2–60B.

NADPH readily gives up the hydride ion it carries in a subsequent oxidation–reduction reaction, because the nicotinamide ring can achieve a more stable arrangement of electrons without it. In this subsequent reaction, which regenerates $NADP^+$, it is the NADPH that is oxidized and the substrate that is reduced. The NADPH is an effective donor of its hydride ion to other molecules

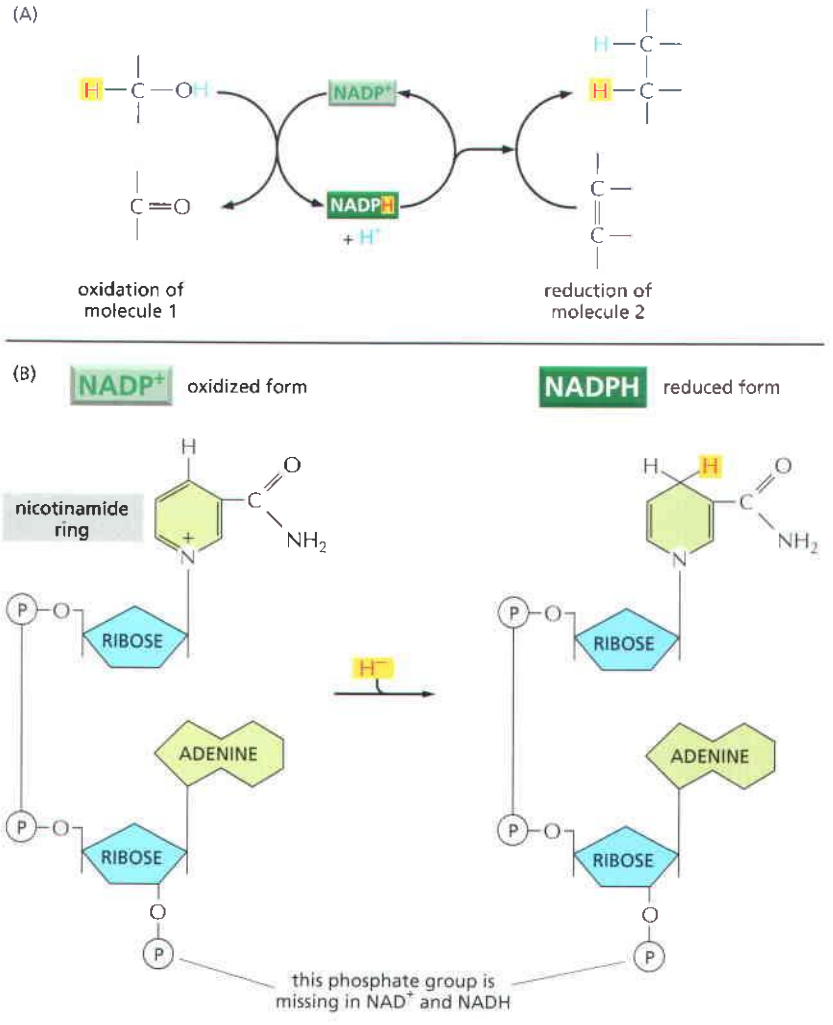


Figure 2–60 NADPH, an important carrier of electrons. (A) NADPH is produced in reactions of the general type shown on the left, in which two hydrogen atoms are removed from a substrate. The oxidized form of the carrier molecule, $NADP^+$, receives one hydrogen atom plus an electron (a hydride ion); the proton (H^+) from the other H atom is released into solution. Because NADPH holds its hydride ion in a high-energy linkage, the added hydride ion can easily be transferred to other molecules, as shown on the right. (B) The structures of $NADP^+$ and NADPH. The part of the $NADP^+$ molecule known as the nicotinamide ring accepts two electrons together with a proton (the equivalent of a hydride ion, H^-), forming NADPH. The molecules NAD^+ and NADH are identical in structure to $NADP^+$ and NADPH, respectively, except that the indicated phosphate group is absent from both.

for the same reason that ATP readily transfers a phosphate: in both cases the transfer is accompanied by a large negative free-energy change. One example of the use of NADPH in biosynthesis is shown in **Figure 2–61**.

The extra phosphate group on NADPH has no effect on the electron-transfer properties of NADPH compared with NADH, being far away from the region involved in electron transfer (see Figure 2–60B). It does, however, give a molecule of NADPH a slightly different shape from that of NADH, making it possible for NADPH and NADH to bind as substrates to completely different sets of enzymes. Thus the two types of carriers are used to transfer electrons (or hydride ions) between two different sets of molecules.

Why should there be this division of labor? The answer lies in the need to regulate two sets of electron-transfer reactions independently. NADPH operates chiefly with enzymes that catalyze anabolic reactions, supplying the high-energy electrons needed to synthesize energy-rich biological molecules. NADH, by contrast, has a special role as an intermediate in the catabolic system of reactions that generate ATP through the oxidation of food molecules, as we will discuss shortly. The genesis of NADH from NAD^+ and that of NADPH from $NADP^+$ occur by different pathways and are independently regulated, so that the cell can adjust the supply of electrons for these two contrasting purposes. Inside the cell the ratio of NAD^+ to NADH is kept high, whereas the ratio of $NADP^+$ to NADPH is kept low. This provides plenty of NAD^+ to act as an oxidizing agent and plenty of NADPH to act as a reducing agent—as required for their special roles in catabolism and anabolism, respectively.

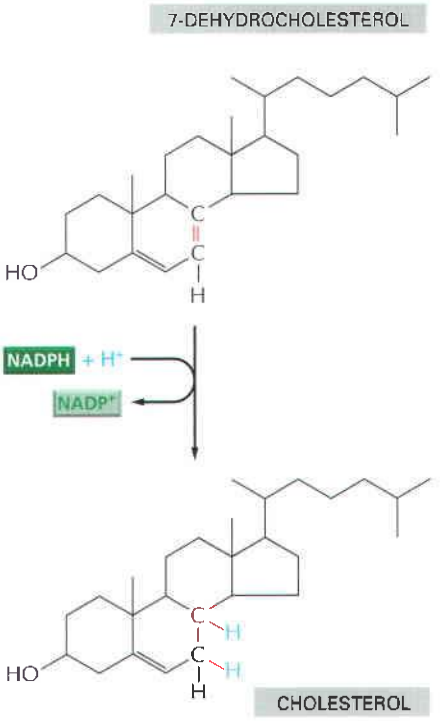


Figure 2–61 The final stage in one of the biosynthetic routes leading to cholesterol. As in many other biosynthetic reactions, the reduction of the C=C bond is achieved by the transfer of a hydride ion from the carrier molecule NADPH, plus a proton (H^+) from the solution.

There Are Many Other Activated Carrier Molecules in Cells

Other activated carriers also pick up and carry a chemical group in an easily transferred, high-energy linkage. For example, coenzyme A carries an acetyl group in a readily transferable linkage, and in this activated form is known as **acetyl CoA** (acetyl coenzyme A). Acetyl CoA (**Figure 2–62**) is used to add two carbon units in the biosynthesis of larger molecules.

In acetyl CoA as in other carrier molecules, the transferable group makes up only a small part of the molecule. The rest consists of a large organic portion that

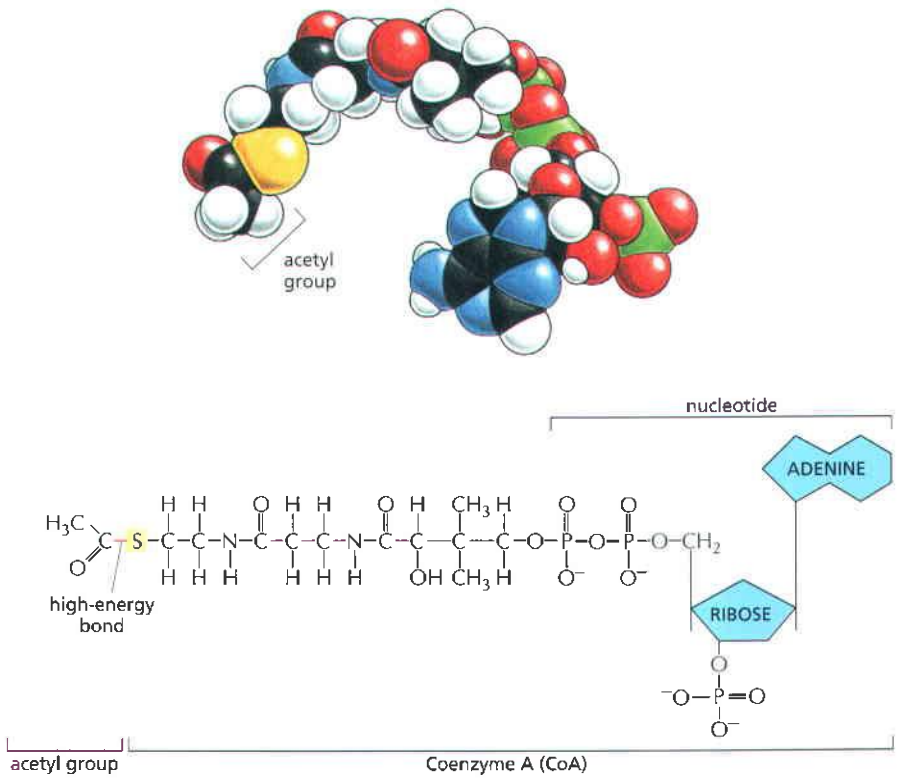


Figure 2–62 The structure of the important activated carrier molecule acetyl CoA. A space-filling model is shown above the structure. The sulfur atom *(yellow)* forms a thioester bond to acetate. Because this is a high-energy linkage, releasing a large amount of free energy when it is hydrolyzed, the acetate molecule can be readily transferred to other molecules.

Table 2–5 Some Activated Carrier Molecules Widely Used in Metabolism

ACTIVATED CARRIER	GROUP CARRIED IN HIGH-ENERGY LINKAGE
ATP	phosphate
NADH, NADPH, $FADH_2$	electrons and hydrogens
Acetyl CoA	acetyl group
Carboxylated biotin	carboxyl group
S-Adenosylmethionine	methyl group
Uridine diphosphate glucose	glucose

serves as a convenient "handle," facilitating the recognition of the carrier molecule by specific enzymes. As with acetyl CoA, this handle portion very often contains a nucleotide (usually adenosine), a curious fact that may be a relic from an early stage of evolution. It is currently thought that the main catalysts for early life-forms—before DNA or proteins—were RNA molecules (or their close relatives), as described in Chapter 6. It is tempting to speculate that many of the carrier molecules that we find today originated in this earlier RNA world, where their nucleotide portions could have been useful for binding them to RNA enzymes.

Figures 2–58 and 2–61 have presented examples of the type of transfer reactions powered by the activated carrier molecules ATP (transfer of phosphate) and NADPH (transfer of electrons and hydrogen). The reactions of other activated carrier molecules involve the transfer of a methyl, carboxyl, or glucose group for the purpose of biosynthesis (Table 2–5). These activated carriers are generated in reactions that are coupled to ATP hydrolysis, as in the example in Figure 2–63. Therefore, the energy that enables their groups to be used for biosynthesis ultimately comes from the catabolic reactions that generate ATP. Similar processes occur in the synthesis of the very large molecules of the cell—the nucleic acids, proteins, and polysaccharides—that we discuss next.

The Synthesis of Biological Polymers Is Driven by ATP Hydrolysis

As discussed previously, the macromolecules of the cell constitute most of its dry mass—that is, of the mass not due to water (see Figure 2–29). These

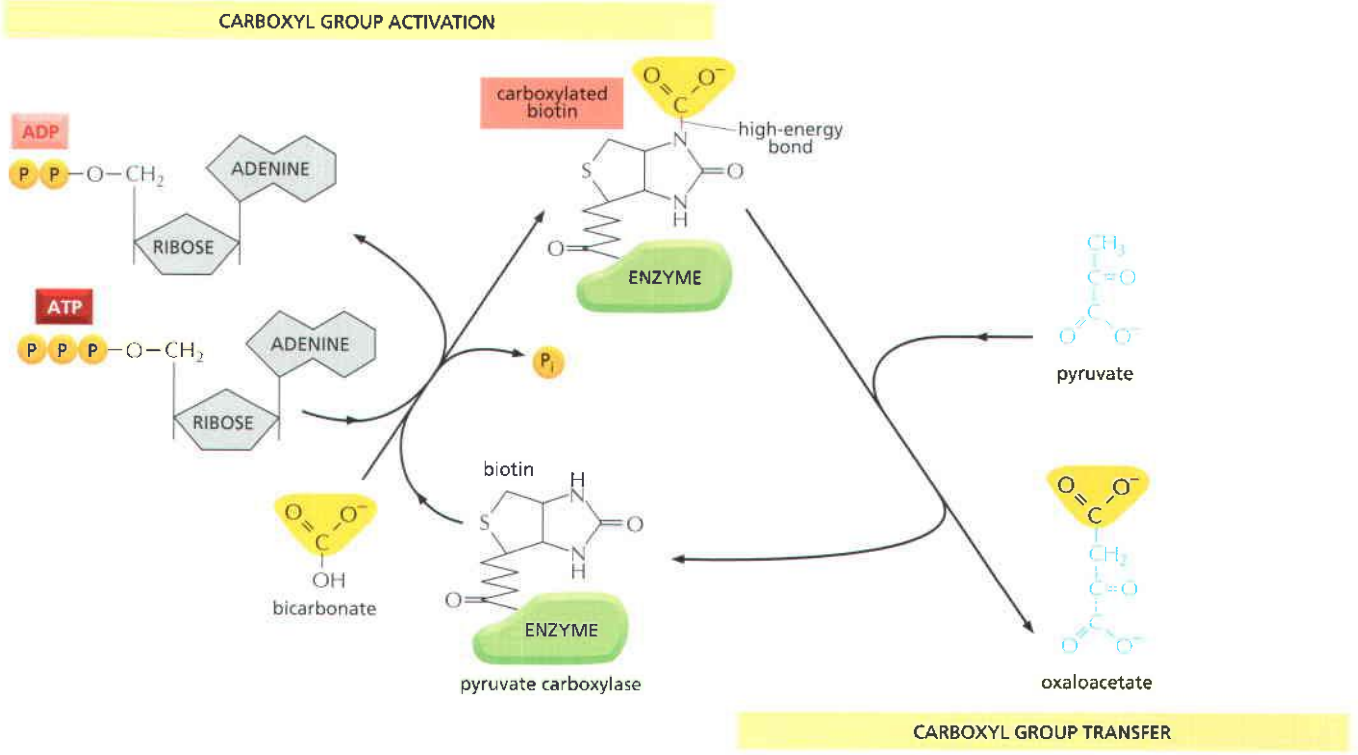


Figure 2–63 A carboxyl group transfer reaction using an activated carrier molecule. Carboxylated biotin is used by the enzyme *pyruvate carboxylase* to transfer a carboxyl group in the production of oxaloacetate, a molecule needed for the citric acid cycle. The acceptor molecule for this group transfer reaction is pyruvate. Other enzymes use biotin to transfer carboxyl groups to other acceptor molecules. Note that synthesis of carboxylated biotin requires energy that is derived from ATP—a general feature of many activated carriers.

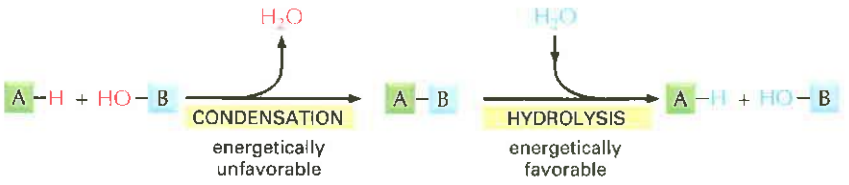


Figure 2–64 Condensation and hydrolysis as opposite reactions. The macromolecules of the cell are polymers that are formed from subunits (or monomers) by a condensation reaction and are broken down by hydrolysis. The condensation reactions are all energetically unfavorable.

molecules are made from subunits (or monomers) that are linked together in a *condensation* reaction, in which the constituents of a water molecule (OH plus H) are removed from the two reactants. Consequently, the reverse reaction—the breakdown of all three types of polymers—occurs by the enzyme-catalyzed addition of water (*hydrolysis*). This hydrolysis reaction is energetically favorable, whereas the biosynthetic reactions require an energy input (**Figure 2–64**).

The nucleic acids (DNA and RNA), proteins, and polysaccharides are all polymers that are produced by the repeated addition of a monomer onto one end of a growing chain. The synthesis reactions for these three types of macromolecules are outlined in **Figure 2–65**. As indicated, the condensation step in each case depends on energy from nucleoside triphosphate hydrolysis. And yet, except for the nucleic acids, there are no phosphate groups left in the final product molecules. How are the reactions that release the energy of ATP hydrolysis coupled to polymer synthesis?

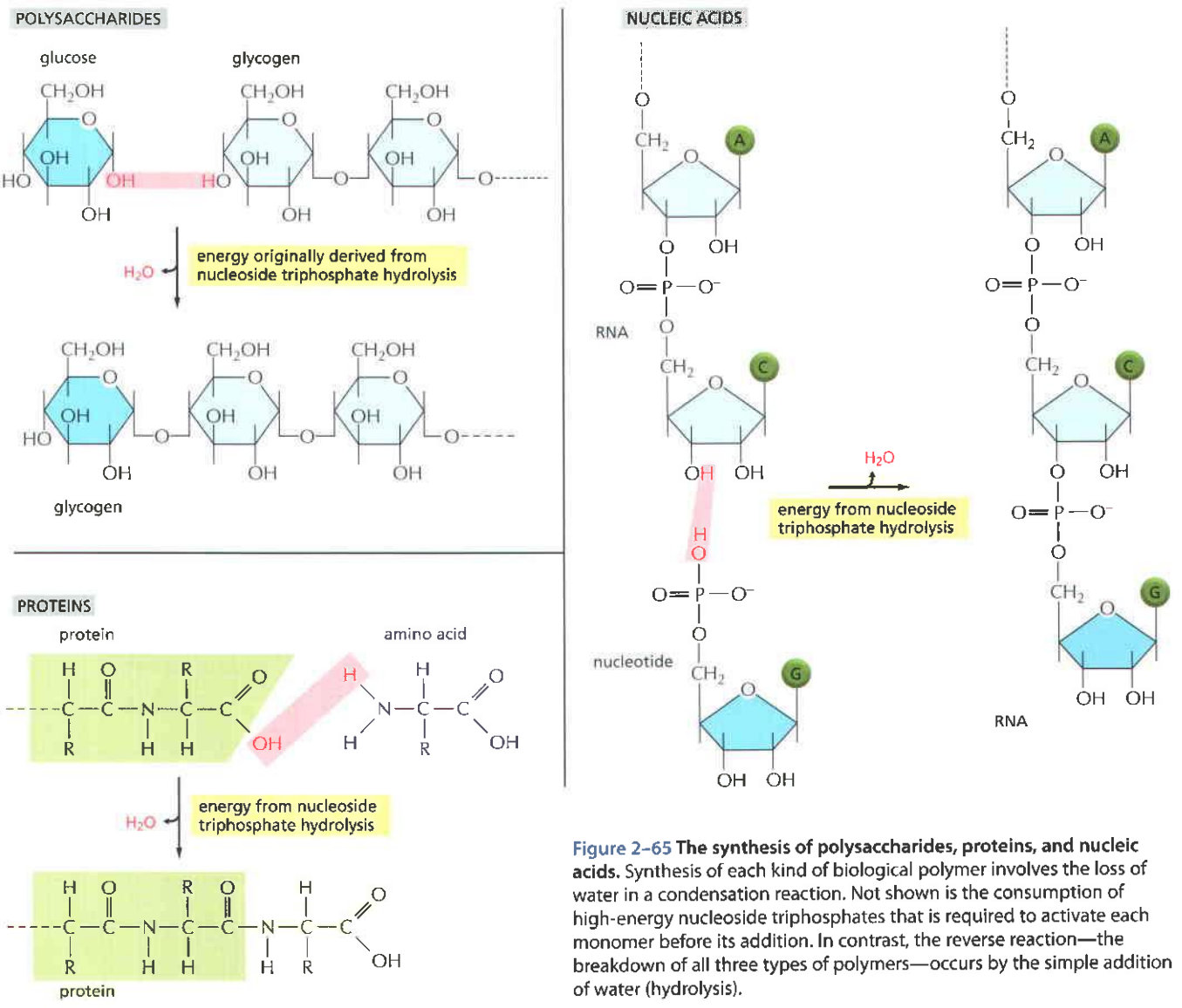


Figure 2–65 The synthesis of polysaccharides, proteins, and nucleic acids. Synthesis of each kind of biological polymer involves the loss of water in a condensation reaction. Not shown is the consumption of high-energy nucleoside triphosphates that is required to activate each monomer before its addition. In contrast, the reverse reaction—the breakdown of all three types of polymers—occurs by the simple addition of water (hydrolysis).

For each type of macromolecule, an enzyme-catalyzed pathway exists which resembles that discussed previously for the synthesis of the amino acid glutamine (see Figure 2–59). The principle is exactly the same, in that the OH group that will be removed in the condensation reaction is first activated by becoming involved in a high-energy linkage to a second molecule. However, the actual mechanisms used to link ATP hydrolysis to the synthesis of proteins and polysaccharides are more complex than that used for glutamine synthesis, since a series of high-energy intermediates is required to generate the final high-energy bond that is broken during the condensation step (discussed in Chapter 6 for protein synthesis).

Each activated carrier has limits in its ability to drive a biosynthetic reaction. The ΔG for the hydrolysis of ATP to ADP and inorganic phosphate (P_i) depends on the concentrations of all of the reactants, but under the usual conditions in a cell it is between –11 and –13 kcal/mole (between –46 and –54 kJ/mole). In principle, this hydrolysis reaction could drive an unfavorable reaction with a ΔG of, perhaps, +10 kcal/mole, provided that a suitable reaction path is available. For some biosynthetic reactions, however, even –13 kcal/mole may not be enough. In these cases the path of ATP hydrolysis can be altered so that it initially produces AMP and pyrophosphate (PP_i), which is itself then hydrolyzed in a subsequent step (**Figure 2–66**). The whole process makes available a total free-energy change of about –26 kcal/mole. An important type of biosynthetic reaction that is driven in this way is the synthesis of nucleic acids (polynucleotides) from nucleoside triphosphates, as illustrated on the right side of **Figure 2–67**.

Note that the repetitive condensation reactions that produce macromolecules can be oriented in one of two ways, giving rise to either the head polymerization or the tail polymerization of monomers. In so-called *head polymerization* the reactive bond required for the condensation reaction is carried on the end of the growing polymer, and it must therefore be regenerated each time that a monomer is added. In this case, each monomer brings with it the reactive bond that will be used in adding the *next* monomer in the series. In *tail polymerization* the reactive bond carried by each monomer is instead used immediately for its own addition (**Figure 2–68**).

We shall see in later chapters that both these types of polymerization are used. The synthesis of polynucleotides and some simple polysaccharides occurs by tail polymerization, for example, whereas the synthesis of proteins occurs by a head polymerization process.

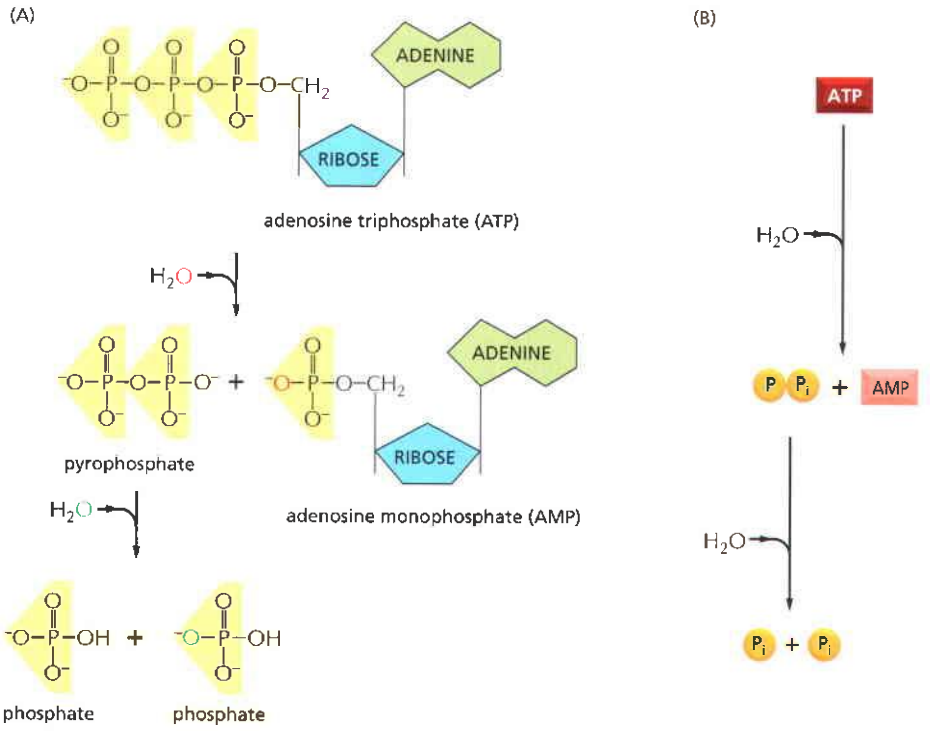


Figure 2–66 An alternative pathway of ATP hydrolysis, in which pyrophosphate is first formed and then hydrolyzed. This route releases about twice as much free energy as the reaction shown earlier in Figure 2–57, and it forms AMP instead of ADP. (A) In the two successive hydrolysis reactions, oxygen atoms from the participating water molecules are retained in the products, as indicated, whereas the hydrogen atoms dissociate to form free hydrogen ions (H^+, not shown). (B) Diagram of overall reaction in summary form.

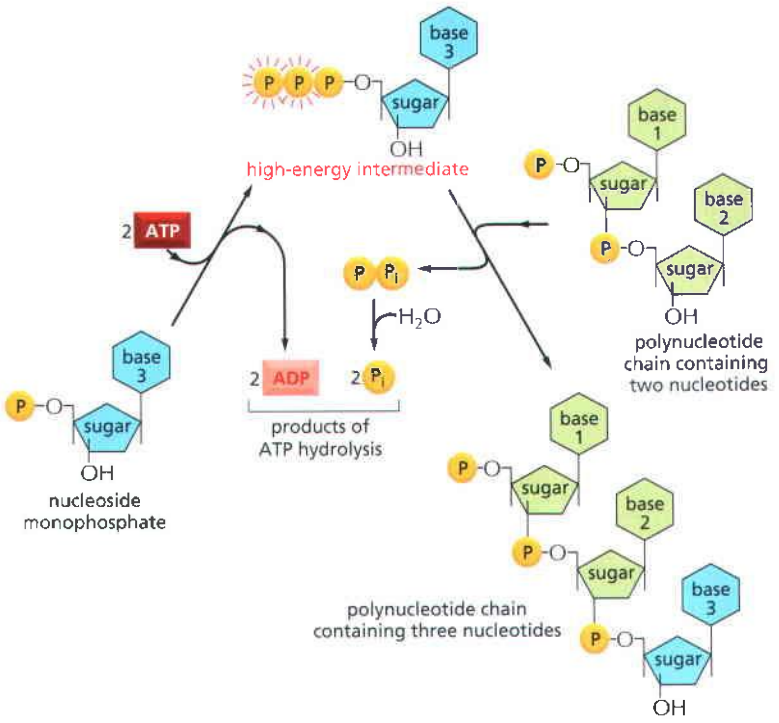


Figure 2–67 Synthesis of a polynucleotide, RNA or DNA, is a multistep process driven by ATP hydrolysis. In the first step, a nucleoside monophosphate is activated by the sequential transfer of the terminal phosphate groups from two ATP molecules. The high-energy intermediate formed—a nucleoside triphosphate—exists free in solution until it reacts with the growing end of an RNA or a DNA chain with release of pyrophosphate. Hydrolysis of the latter to inorganic phosphate is highly favorable and helps to drive the overall reaction in the direction of polynucleotide synthesis. For details, see Chapter 5.

Summary

Living cells are highly ordered and need to create order within themselves to survive and grow. This is thermodynamically possible only because of a continual input of energy, part of which must be released from the cells to their environment as heat. The energy comes ultimately from the electromagnetic radiation of the sun, which drives the formation of organic molecules in photosynthetic organisms such as green plants. Animals obtain their energy by eating these organic molecules and oxidizing them in a series of enzyme-catalyzed reactions that are coupled to the formation of ATP—a common currency of energy in all cells.

To make possible the continual generation of order in cells, the energetically favorable hydrolysis of ATP is coupled to energetically unfavorable reactions. In the biosynthesis of macromolecules, this is accomplished by the transfer of phosphate groups to form reactive phosphorylated intermediates. Because the energetically unfavorable reaction now becomes energetically favorable, ATP hydrolysis is said to drive the reaction. Polymeric molecules such as proteins, nucleic acids, and polysaccharides are assembled from small activated precursor molecules by repetitive condensation reactions that are driven in this way. Other reactive molecules, called either active carriers or coenzymes, transfer other chemical groups in the course of biosynthesis: NADPH transfers hydrogen as a proton plus two electrons (a hydride ion), for example, whereas acetyl CoA transfers an acetyl group.

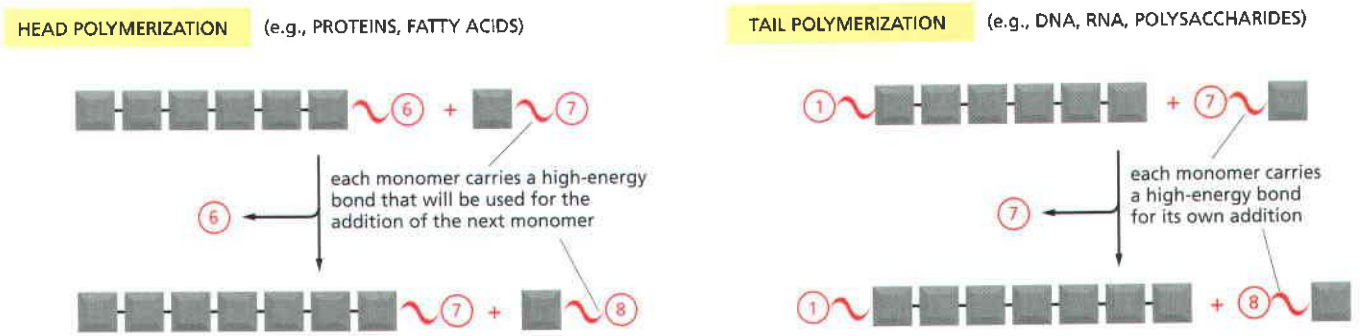


Figure 2–68 The orientation of the active intermediates in the repetitive condensation reactions that form biological polymers. The head growth of polymers is compared with its alternative, tail growth. As indicated, these two mechanisms are used to produce different types of biological macromolecules.

HOW CELLS OBTAIN ENERGY FROM FOOD

The constant supply of energy that cells need to generate and maintain the biological order that keeps them alive comes from the chemical bond energy in food molecules, which thereby serve as fuel for cells.

The proteins, lipids, and polysaccharides that make up most of the food we eat must be broken down into smaller molecules before our cells can use them—either as a source of energy or as building blocks for other molecules. Enzymatic digestion breaks down the large polymeric molecules in food into their monomer subunits—proteins into amino acids, polysaccharides into sugars, and fats into fatty acids and glycerol. After digestion, the small organic molecules derived from food enter the cytosol of cells, where their gradual oxidation begins.

Sugars are particularly important fuel molecules, and they are oxidized in small controlled steps to carbon dioxide (CO_2) and water (**Figure 2–69**). In this section we trace the major steps in the breakdown, or catabolism, of sugars and show how they produce ATP, NADH, and other activated carrier molecules in animal cells. A very similar pathway also operates in plants, fungi, and many bacteria. As we shall see, the oxidation of fatty acids is equally important for cells. Other molecules, such as proteins, can also serve as energy sources when they are funneled through appropriate enzymatic pathways.

Glycolysis Is a Central ATP-Producing Pathway

The major process for oxidizing sugars is the sequence of reactions known as **glycolysis**—from the Greek *glukus*, "sweet," and *lusis*, "rupture." Glycolysis produces ATP without the involvement of molecular oxygen (O_2 gas). It occurs in the cytosol of most cells, including many anaerobic microorganisms (those that can live without using molecular oxygen). Glycolysis probably evolved early in the history of life, before photosynthetic organisms introduced oxygen into the atmosphere. During glycolysis, a glucose molecule with six carbon atoms is converted into two molecules of *pyruvate*, each of which contains three carbon atoms. For each glucose molecule, two molecules of ATP are hydrolyzed to provide energy to drive the early steps, but four molecules of ATP are produced in the later steps. At the end of glycolysis, there is consequently a net gain of two molecules of ATP for each glucose molecule broken down.

The glycolytic pathway is outlined in **Figure 2–70** and shown in more detail in Panel 2–8 (pp. 120–121). Glycolysis involves a sequence of 10 separate reactions, each producing a different sugar intermediate and each catalyzed by a

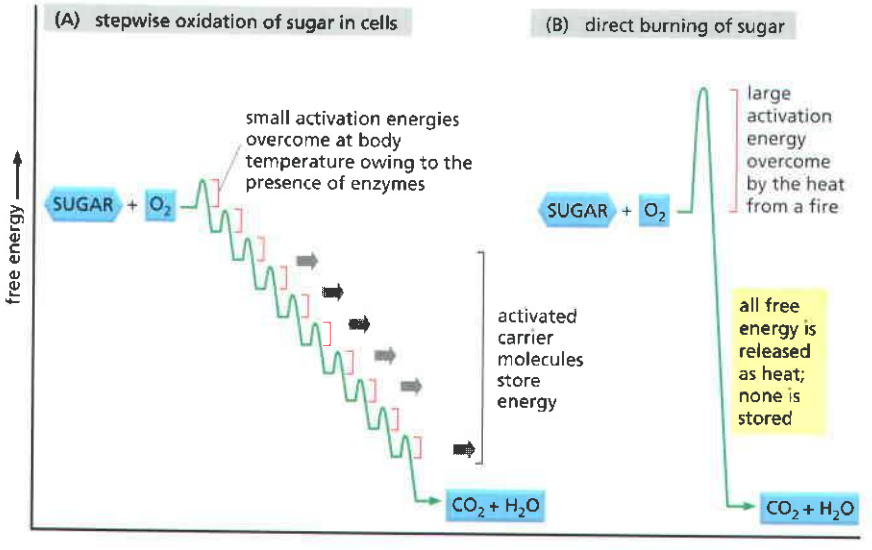


Figure 2–69 Schematic representation of the controlled stepwise oxidation of sugar in a cell, compared with ordinary burning. (A) In the cell, enzymes catalyze oxidation via a series of small steps in which free energy is transferred in conveniently sized packets to carrier molecules—most often ATP and NADH. At each step, an enzyme controls the reaction by reducing the activation energy barrier that has to be surmounted before the specific reaction can occur. The total free energy released is exactly the same in (A) and (B). But if the sugar were instead oxidized to CO_2 and H_2O in a single step, as in (B), it would release an amount of energy much larger than could be captured for useful purposes.

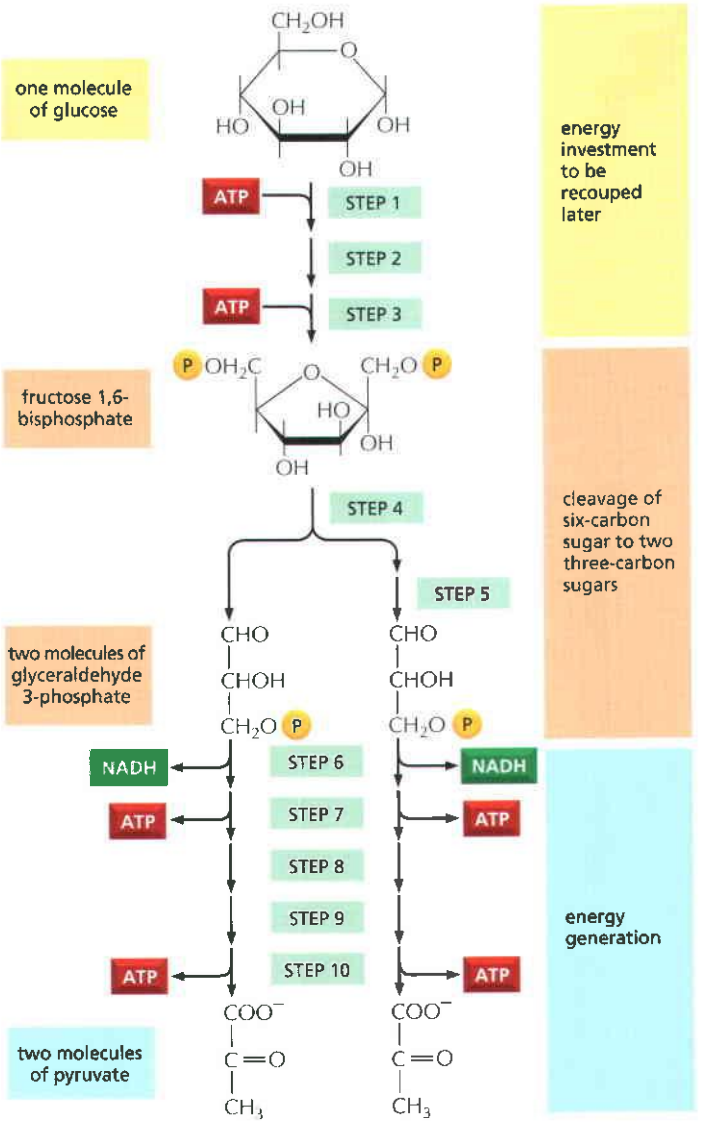


Figure 2–70 An outline of glycolysis. <GGGC> Each of the 10 steps shown is catalyzed by a different enzyme. Note that step 4 cleaves a six-carbon sugar into two three-carbon sugars, so that the number of molecules at every stage after this doubles. As indicated, step 6 begins the energy generation phase of glycolysis. Because two molecules of ATP are hydrolyzed in the early, energy investment phase, glycolysis results in the net synthesis of 2 ATP and 2 NADH molecules per molecule of glucose (see also Panel 2–8).

different enzyme. Like most enzymes, these have names ending in *ase*—such as isomer*ase* and dehydrogen*ase*—to indicate the type of reaction they catalyze.

Although no molecular oxygen is used in glycolysis, oxidation occurs, in that electrons are removed by NAD^+ (producing NADH) from some of the carbons derived from the glucose molecule. The stepwise nature of the process releases the energy of oxidation in small packets, so that much of it can be stored in activated carrier molecules rather than all of it being released as heat (see Figure 2–69). Thus, some of the energy released by oxidation drives the direct synthesis of ATP molecules from ADP and P_i, and some remains with the electrons in the high-energy electron carrier NADH.

Two molecules of NADH are formed per molecule of glucose in the course of glycolysis. In aerobic organisms (those that require molecular oxygen to live), these NADH molecules donate their electrons to the electron-transport chain described in Chapter 14, and the NAD^+ formed from the NADH is used again for glycolysis (see step 6 in Panel 2–8, pp. 120–121).

Fermentations Produce ATP in the Absence of Oxygen

For most animal and plant cells, glycolysis is only a prelude to the final stage of the breakdown of food molecules. In these cells, the pyruvate formed by glycolysis is

rapidly transported into the mitochondria, where it is converted into CO_2 plus acetyl CoA, which is then completely oxidized to CO_2 and H_2O.

In contrast, for many anaerobic organisms—which do not utilize molecular oxygen and can grow and divide without it—glycolysis is the principal source of the cell's ATP. This is also true for certain animal tissues, such as skeletal muscle, that can continue to function when molecular oxygen is limiting. In these anaerobic conditions, the pyruvate and the NADH electrons stay in the cytosol. The pyruvate is converted into products excreted from the cell—for example, into ethanol and CO_2 in the yeasts used in brewing and breadmaking, or into lactate in muscle. In this process, the NADH gives up its electrons and is converted back into NAD^+. This regeneration of NAD^+ is required to maintain the reactions of glycolysis (**Figure 2–71**).

Anaerobic energy-yielding pathways like these are called **fermentations**. Studies of the commercially important fermentations carried out by yeasts inspired much of early biochemistry. Work in the nineteenth century led in 1896 to the then startling recognition that these processes could be studied outside living organisms, in cell extracts. This revolutionary discovery eventually made it possible to dissect out and study each of the individual reactions in the fermentation process. The piecing together of the complete glycolytic pathway in the 1930s was a major triumph of biochemistry, and it was quickly followed by the recognition of the central role of ATP in cell processes. Thus, most of the fundamental concepts discussed in this chapter have been understood for many years.

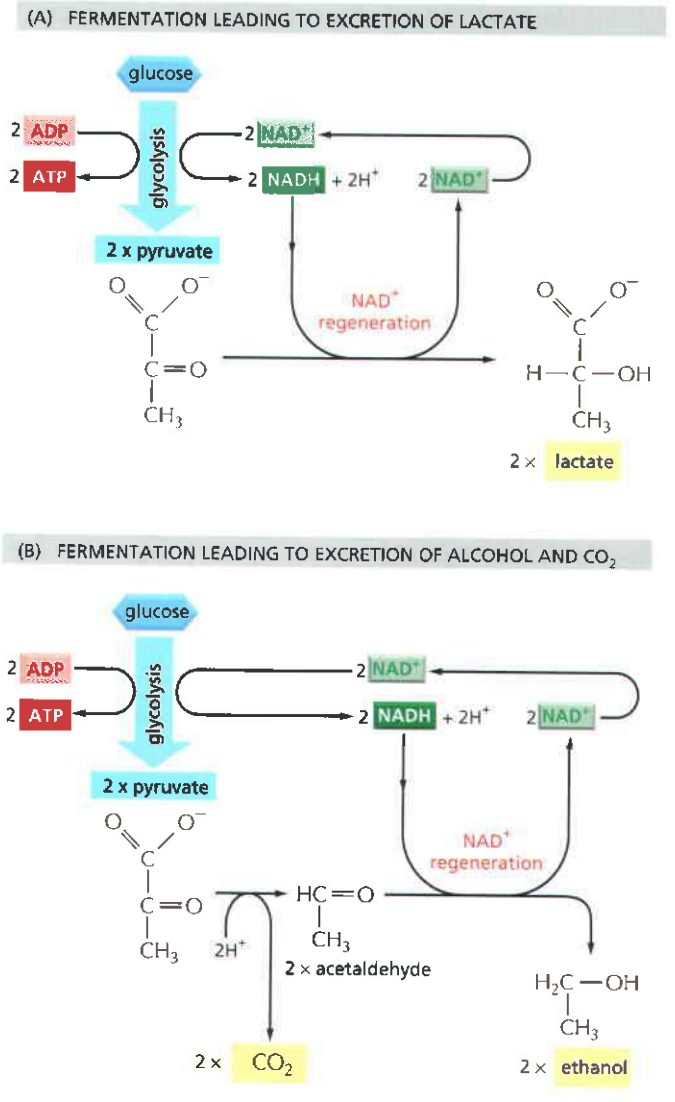


Figure 2–71 Two pathways for the anaerobic breakdown of pyruvate. (A) When there is inadequate oxygen, for example, in a muscle cell undergoing vigorous contraction, the pyruvate produced by glycolysis is converted to lactate as shown. This reaction regenerates the NAD^+ consumed in step 6 of glycolysis, but the whole pathway yields much less energy overall than complete oxidation. (B) In some organisms that can grow anaerobically, such as yeasts, pyruvate is converted via acetaldehyde into carbon dioxide and ethanol. Again, this pathway regenerates NAD^+ from NADH, as required to enable glycolysis to continue. Both (A) and (B) are examples of *fermentations*.

Glycolysis Illustrates How Enzymes Couple Oxidation to Energy Storage

Returning to the paddle-wheel analogy that we used to introduce coupled reactions (see Figure 2–56), we can now equate enzymes with the paddle wheel. Enzymes act to harvest useful energy from the oxidation of organic molecules by coupling an energetically unfavorable reaction with a favorable one. To demonstrate this coupling, we examine a step in glycolysis to see exactly how such coupled reactions occur.

Two central reactions in glycolysis (steps 6 and 7) convert the three-carbon sugar intermediate glyceraldehyde 3-phosphate (an aldehyde) into 3-phosphoglycerate (a carboxylic acid; see Panel 2–8, pp. 120–121). This entails the oxidation of an aldehyde group to a carboxylic acid group in a reaction that occurs in two steps. The overall reaction releases enough free energy to convert a molecule of ADP to ATP and to transfer two electrons from the aldehyde to NAD^+ to form NADH, while still releasing enough heat to the environment to make the overall reaction energetically favorable (ΔG° for the overall reaction is –3.0 kcal/mole).

Figure 2–72 outlines the means by which this remarkable feat of energy harvesting is accomplished. The indicated chemical reactions are precisely guided by two enzymes to which the sugar intermediates are tightly bound. In fact, as detailed in Figure 2–72, the first enzyme (glyceraldehyde 3-phosphate dehydrogenase) forms a short-lived covalent bond to the aldehyde through a reactive –SH group on the enzyme, and catalyzes its oxidation by NAD^+ in this attached state. The reactive enzyme–substrate bond is then displaced by an inorganic phosphate ion to produce a high-energy phosphate intermediate, which is released from the enzyme. This intermediate binds to the second enzyme (phosphoglycerate kinase), which catalyzes the energetically favorable transfer of the high-energy phosphate just created to ADP, forming ATP and completing the process of oxidizing an aldehyde to a carboxylic acid.

We have shown this particular oxidation process in some detail because it provides a clear example of enzyme-mediated energy storage through coupled reactions (**Figure 2–73**). Steps 6 and 7 are the only reactions in glycolysis that create a high-energy phosphate linkage directly from inorganic phosphate. As such, they account for the net yield of two ATP molecules and two NADH molecules per molecule of glucose (see Panel 2–8, pp. 120–121).

As we have just seen, ATP can be formed readily from ADP when a reaction intermediate is formed with a phosphate bond of higher-energy than the phosphate bond in ATP. Phosphate bonds can be ordered in energy by comparing the standard free-energy change (ΔG°) for the breakage of each bond by hydrolysis. **Figure 2–74** compares the high-energy phosphoanhydride bonds in ATP with the energy of some other phosphate bonds, several of which are generated during glycolysis.

Organisms Store Food Molecules in Special Reservoirs

All organisms need to maintain a high ATP/ADP ratio to maintain biological order in their cells. Yet animals have only periodic access to food, and plants need to survive overnight without sunlight, when they are unable to produce sugar from photosynthesis. For this reason, both plants and animals convert sugars and fats to special forms for storage (**Figure 2–75**).

To compensate for long periods of fasting, animals store fatty acids as fat droplets composed of water-insoluble triacylglycerols, largely in the cytoplasm of specialized fat cells, called adipocytes. For shorter-term storage, sugar is stored as glucose subunits in the large branched polysaccharide **glycogen**, which is present as small granules in the cytoplasm of many cells, including liver and muscle. The synthesis and degradation of glycogen are rapidly regulated according to need. When cells need more ATP than they can generate from the food molecules taken in from the bloodstream, they break down glycogen in a reaction that produces glucose 1-phosphate, which is rapidly converted to glucose 6-phosphate for glycolysis.

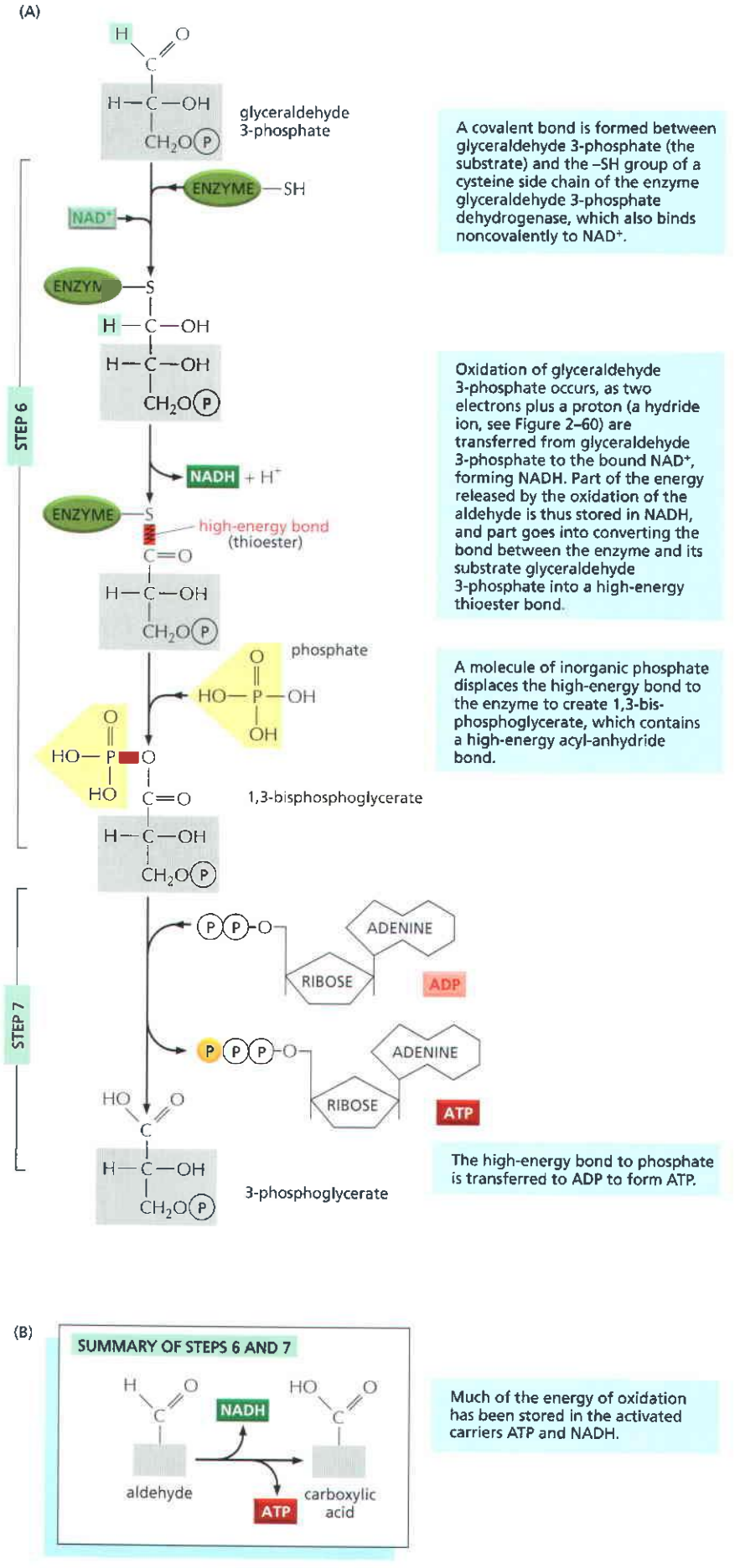


Figure 2–72 Energy storage in steps 6 and 7 of glycolysis. In these steps the oxidation of an aldehyde to a carboxylic acid is coupled to the formation of ATP and NADH. (A) Step 6 begins with the formation of a covalent bond between the substrate (glyceraldehyde 3-phosphate) and an –SH group exposed on the surface of the enzyme (glyceraldehyde 3-phosphate dehydrogenase). The enzyme then catalyzes transfer of hydrogen (as a hydride ion—a proton plus two electrons) from the bound glyceraldehyde 3-phosphate to a molecule of NAD^+. Part of the energy released in this oxidation is used to form a molecule of NADH and part is used to convert the original linkage between the enzyme and its substrate to a high-energy thioester bond (shown in *red*). A molecule of inorganic phosphate then displaces this high-energy bond on the enzyme, creating a high-energy sugar-phosphate bond instead *(red)*. At this point the enzyme has not only stored energy in NADH, but also coupled the energetically favorable oxidation of an aldehyde to the energetically unfavorable formation of a high-energy phosphate bond. The second reaction has been driven by the first, thereby acting like the "paddle-wheel" coupler in Figure 2–56.

In reaction step 7, the high-energy sugar-phosphate intermediate just made, 1,3-bisphosphoglycerate, binds to a second enzyme, phosphoglycerate kinase. The reactive phosphate is transferred to ADP, forming a molecule of ATP and leaving a free carboxylic acid group on the oxidized sugar.

(B) Summary of the overall chemical change produced by reactions 6 and 7.

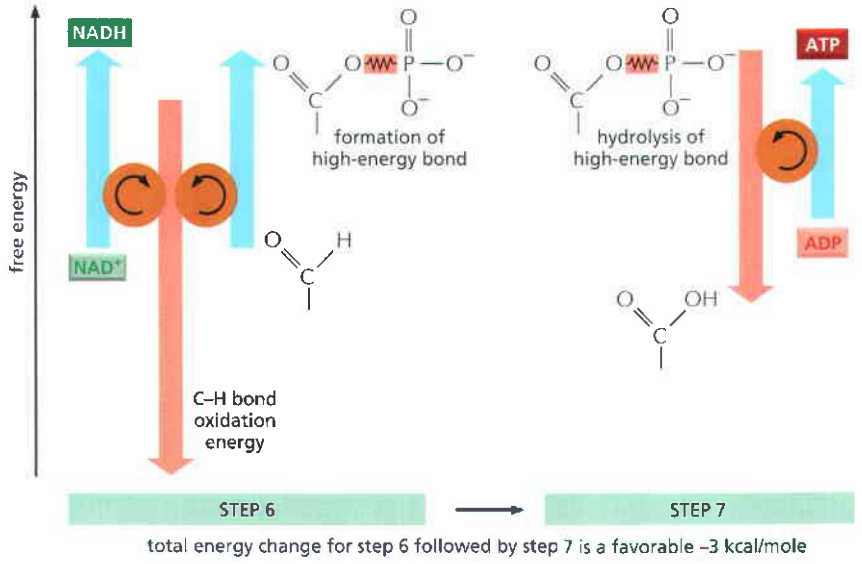


Figure 2–73 Schematic view of the coupled reactions that form NADH and ATP in steps 6 and 7 of glycolysis. The C–H bond oxidation energy drives the formation of both NADH and a high-energy phosphate bond. The breakage of the high-energy bond then drives ATP formation.

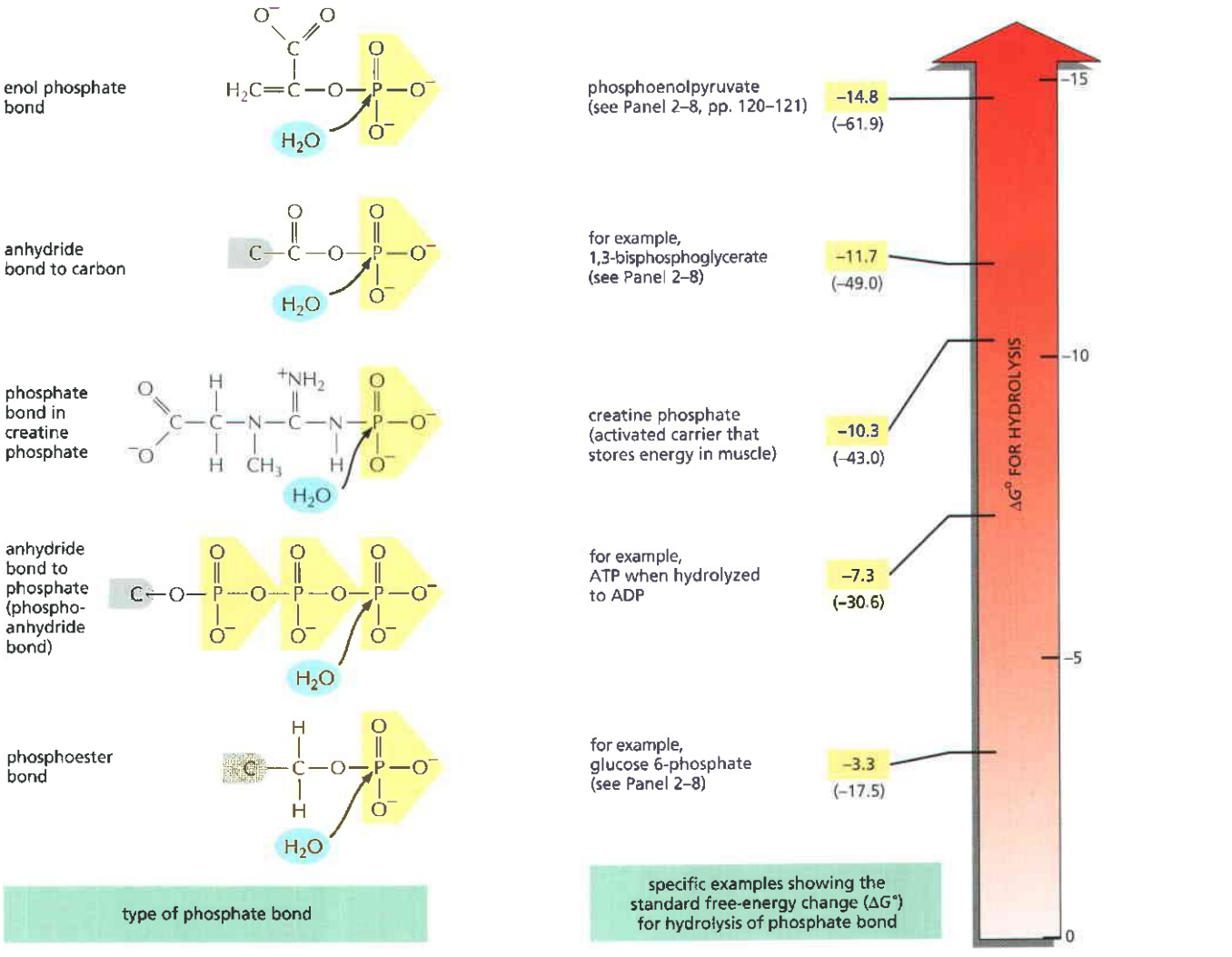
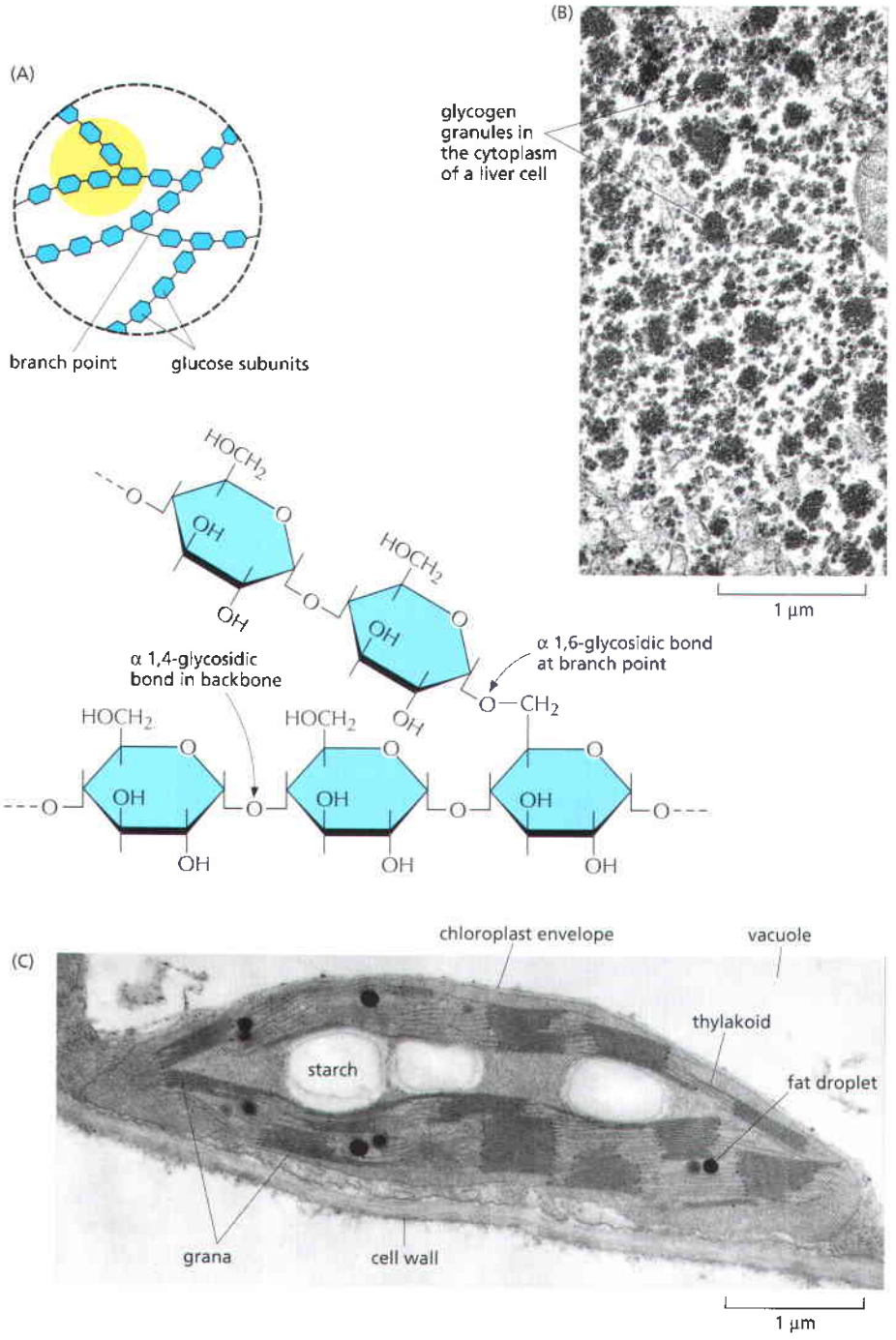


Figure 2–74 Phosphate bonds have different energies. Examples of different types of phosphate bonds with their sites of hydrolysis are shown in the molecules depicted on the left. Those starting with a *gray* carbon atom show only part of a molecule. Examples of molecules containing such bonds are given on the right, with the free-energy change for hydrolysis in kilocalories (kilojoules in parentheses). The transfer of a phosphate group from one molecule to another is energetically favorable if the standard free-energy change (ΔG°) for hydrolysis of the phosphate bond of the first molecule is more negative than that for hydrolysis of the phosphate bond in the second. Thus, a phosphate group is readily transferred from 1,3-bisphosphoglycerate to ADP to form ATP. The hydrolysis reaction can be viewed as the transfer of the phosphate group to water.



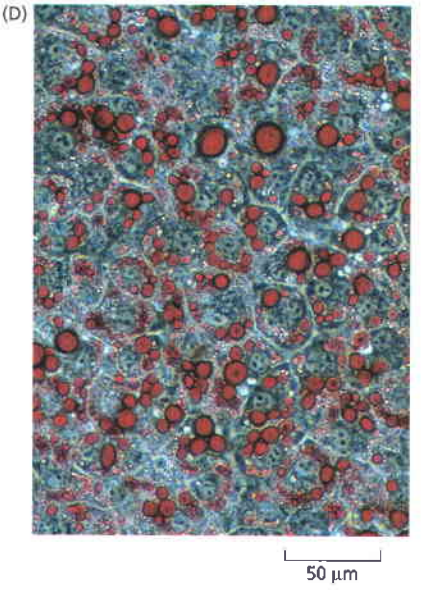


Figure 2–75 The storage of sugars and fats in animal and plant cells. (A) The structures of starch and glycogen, the storage form of sugars in plants and animals, respectively. Both are storage polymers of the sugar glucose and differ only in the frequency of branch points (the region in *yellow* is shown enlarged below). There are many more branches in glycogen than in starch. (B) An electron micrograph shows glycogen granules in the cytoplasm of a liver cell. (C) A thin section of a single chloroplast from a plant cell, showing the starch granules and lipid (fat droplets) that have accumulated as a result of the biosyntheses occurring there. (D) Fat droplets (stained *red*) beginning to accumulate in developing fat cells of an animal. (B, courtesy of Robert Fletterick and Daniel S. Friend; C, courtesy of K. Plaskitt; D, courtesy of Ronald M. Evans and Peter Totonoz.)

Quantitatively, **fat** is far more important than glycogen as an energy store for animals, presumably because it provides for more efficient storage. The oxidation of a gram of fat releases about twice as much energy as the oxidation of a gram of glycogen. Moreover, glycogen differs from fat in binding a great deal of water, producing a sixfold difference in the actual mass of glycogen required to store the same amount of energy as fat. An average adult human stores enough glycogen for only about a day of normal activities but enough fat to last for nearly a month. If our main fuel reservoir had to be carried as glycogen instead of fat, body weight would increase by an average of about 60 pounds.

Although plants produce NADPH and ATP by photosynthesis, this important process occurs in a specialized organelle, called a chloroplast, which is isolated from the rest of the plant cell by a membrane that is impermeable to both types of activated carrier molecules. Moreover, the plant contains many other cells—such as those in the roots—that lack chloroplasts and therefore cannot produce their own sugars. Therefore, for most of its ATP production, the plant relies on an

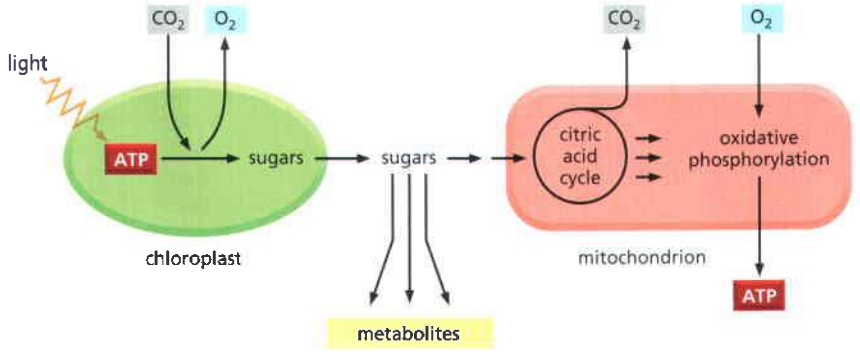


Figure 2–76 How the ATP needed for most plant cell metabolism is made. In plants, the chloroplasts and mitochondria collaborate to supply cells with metabolites and ATP. (For details, see Chapter 14.)

export of sugars from its chloroplasts to the mitochondria that are located in all cells of the plant. Most of the ATP needed by the plant is synthesized in these mitochondria and exported from them to the rest of the plant cell, using exactly the same pathways for the oxidative breakdown of sugars as in nonphotosynthetic organisms (**Figure 2–76**).

During periods of excess photosynthetic capacity during the day, chloroplasts convert some of the sugars that they make into fats and into **starch**, a polymer of glucose analogous to the glycogen of animals. The fats in plants are triacylglycerols, just like the fats in animals, and differ only in the types of fatty acids that predominate. Fat and starch are both stored in the chloroplast as reservoirs to be mobilized as an energy source during periods of darkness (see Figure 2–75C).

The embryos inside plant seeds must live on stored sources of energy for a prolonged period, until they germinate to produce leaves that can harvest the energy in sunlight. For this reason plant seeds often contain especially large amounts of fats and starch—which makes them a major food source for animals, including ourselves (**Figure 2–77**).

Most Animal Cells Derive Their Energy from Fatty Acids Between Meals

After a meal, most of the energy that an animal needs is derived from sugars derived from food. Excess sugars, if any, are used to replenish depleted glycogen stores, or to synthesize fats as a food store. But soon the fat stored in adipose tissue is called into play, and by the morning after an overnight fast, fatty acid oxidation generates most of the ATP we need.

Low glucose levels in the blood trigger the breakdown of fats for energy production. As illustrated in **Figure 2–78**, the triacylglycerols stored in fat droplets in adipocytes are hydrolyzed to produce fatty acids and glycerol, and the fatty acids released are transferred to cells in the body through the bloodstream. While animals readily convert sugars to fats, they cannot convert fatty acids to sugars. Instead, the fatty acids are oxidized directly.



Figure 2–77 Some plant seeds that serve as important foods for humans. Corn, nuts, and peas all contain rich stores of starch and fat that provide the young plant embryo in the seed with energy and building blocks for biosynthesis. (Courtesy of the John Innes Foundation.)

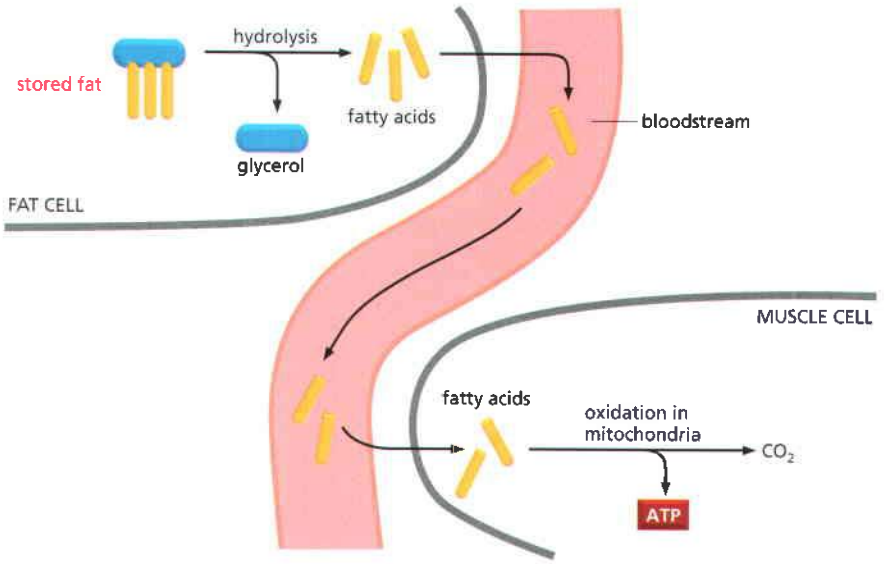


Figure 2–78 How stored fats are mobilized for energy production in animals. Low glucose levels in the blood trigger the hydrolysis of the triacylglycerol molecules in fat droplets to free fatty acids and glycerol, as illustrated. These fatty acids enter the bloodstream, where they bind to the abundant blood protein, serum albumin. Special fatty acid transporters in the plasma membrane of cells that oxidize fatty acids, such as muscle cells, then pass these fatty acids into the cytosol, from which they are moved into mitochondria for energy production (see Figure 2–80).

Sugars and Fats Are Both Degraded to Acetyl CoA in Mitochondria

In aerobic metabolism, the pyruvate that was produced by glycolysis from sugars in the cytosol is transported into the *mitochondria* of eucaryotic cells. There, it is rapidly decarboxylated by a giant complex of three enzymes, called the *pyruvate dehydrogenase complex*. The products of pyruvate decarboxylation are a molecule of CO_2 (a waste product), a molecule of NADH, and acetyl CoA (Figure 2–79).

The fatty acids imported from the bloodstream are moved into mitochondria, where all of their oxidation takes place (Figure 2–80). Each molecule of fatty acid (as the activated molecule *fatty acyl CoA*) is broken down completely by a cycle of reactions that trims two carbons at a time from its carboxyl end, generating one molecule of acetyl CoA for each turn of the cycle. A molecule of NADH and a molecule of $FADH_2$ are also produced in this process (Figure 2–81).

Sugars and fats are the major energy sources for most non-photosynthetic organisms, including humans. However, most of the useful energy that can be

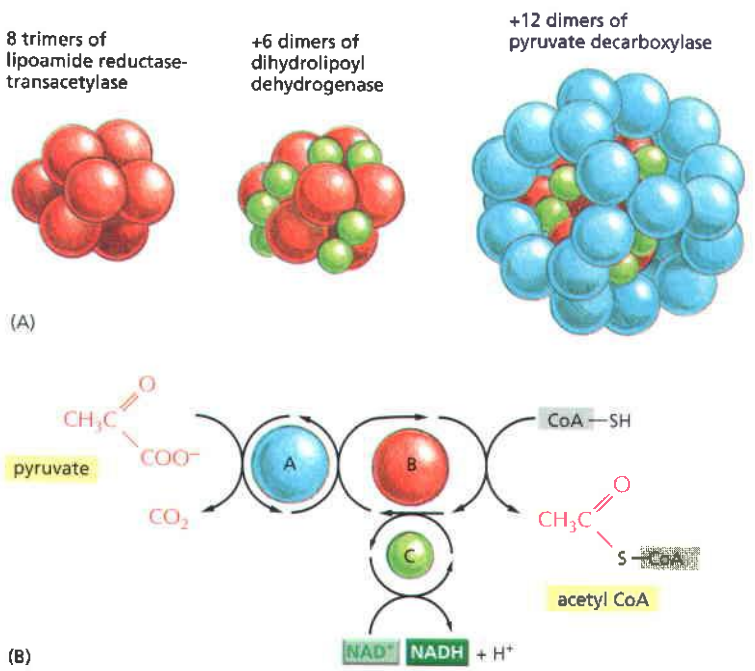


Figure 2–79 The oxidation of pyruvate to acetyl CoA and CO_2. (A) The structure of the pyruvate dehydrogenase complex, which contains 60 polypeptide chains. This is an example of a large multienzyme complex in which reaction intermediates are passed directly from one enzyme to another. In eucaryotic cells it is located in the mitochondrion. (B) The reactions carried out by the pyruvate dehydrogenase complex. The complex converts pyruvate to acetyl CoA in the mitochondrial matrix; NADH is also produced in this reaction. A, B, and C are the three enzymes *pyruvate decarboxylase*, *lipoamide reductase-transacetylase*, and *dihydrolipoyl dehydrogenase*, respectively. These enzymes are illustrated in (A); their activities are linked as shown.

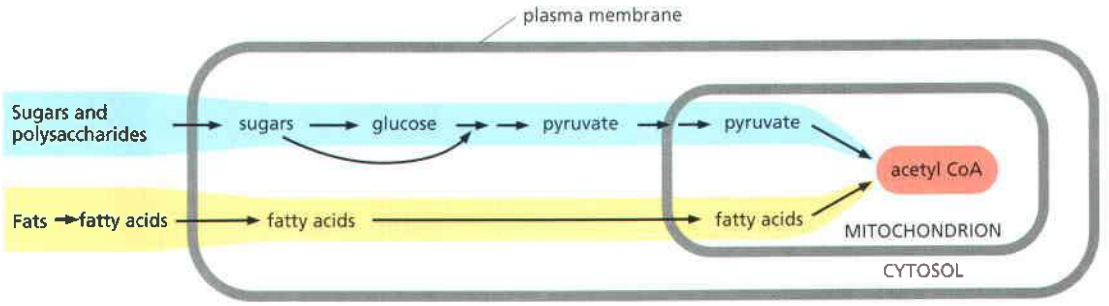


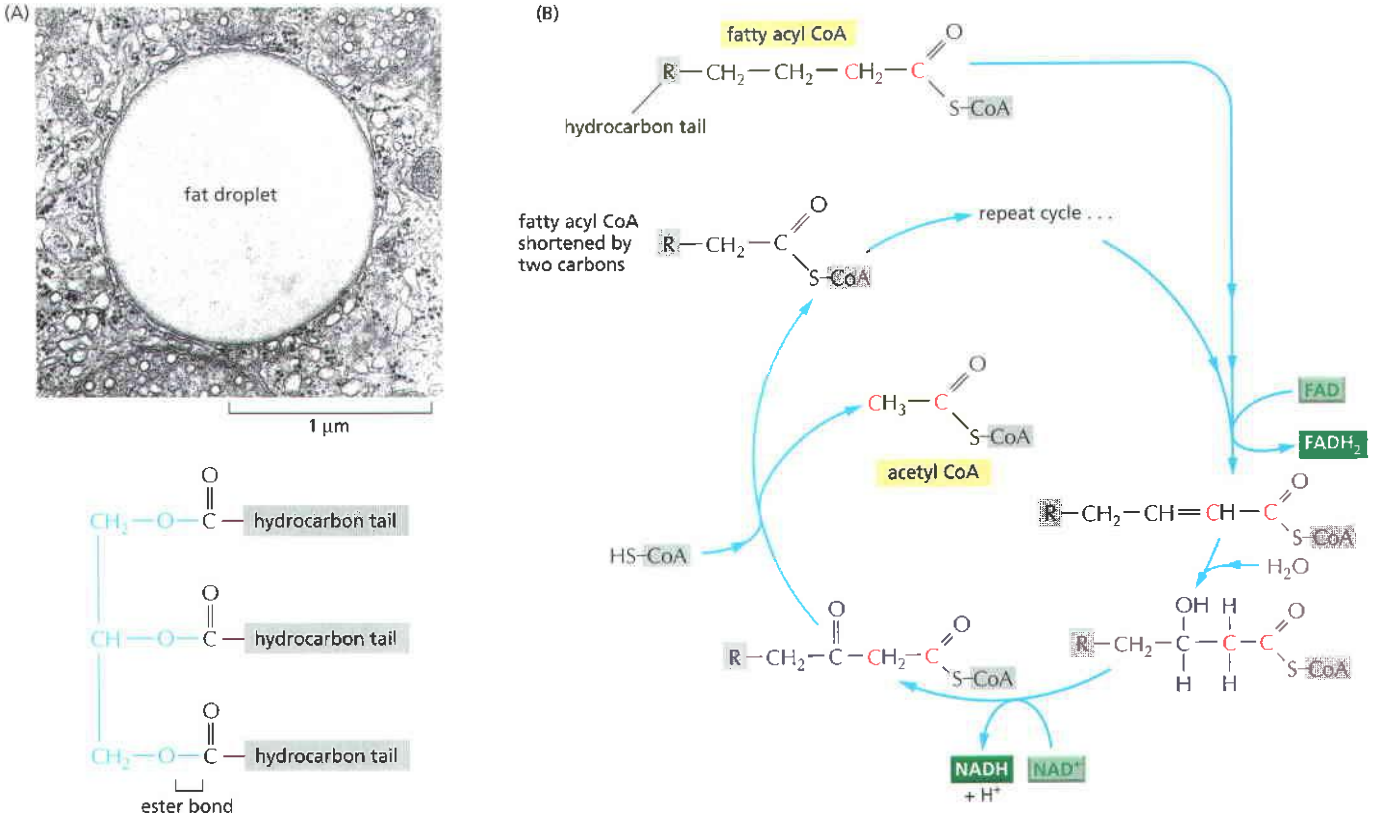
Figure 2–80 Pathways for the production of acetyl CoA from sugars and fats. The mitochondrion in eucaryotic cells is the place where acetyl CoA is produced from both types of major food molecules. It is therefore the place where most of the cell's oxidation reactions occur and where most of its ATP is made. The structure and function of mitochondria are discussed in detail in Chapter 14.

extracted from the oxidation of both types of foodstuffs remains stored in the acetyl CoA molecules that are produced by the two types of reactions just described. The citric acid cycle of reactions, in which the acetyl group in acetyl CoA is oxidized to CO_2 and H_2O, is therefore central to the energy metabolism of aerobic organisms. In eucaryotes these reactions all take place in mitochondria. We should therefore not be surprised to discover that the mitochondrion is the place where most of the ATP is produced in animal cells. In contrast, aerobic bacteria carry out all of their reactions in a single compartment, the cytosol, and it is here that the citric acid cycle takes place in these cells.

The Citric Acid Cycle Generates NADH by Oxidizing Acetyl Groups to CO_2

In the nineteenth century, biologists noticed that in the absence of air (anaerobic conditions) cells produce lactic acid (for example, in muscle) or ethanol (for example, in yeast), while in its presence (aerobic conditions) they consume O_2 and produce CO_2 and H_2O. Efforts to define the pathways of aerobic metabolism

Figure 2–81 The oxidation of fatty acids to acetyl CoA. (A) Electron micrograph of a lipid droplet in the cytoplasm *(top)*, and the structure of fats *(bottom)*. Fats are triacylglycerols. The glycerol portion, to which three fatty acids are linked through ester bonds, is shown here in *blue*. Fats are insoluble in water and form large lipid droplets in the specialized fat cells (called adipocytes) in which they are stored. (B) The fatty acid oxidation cycle. The cycle is catalyzed by a series of four enzymes in the mitochondrion. Each turn of the cycle shortens the fatty acid chain by two carbons (shown in *red*) and generates one molecule of acetyl CoA and one molecule each of NADH and $FADH_2$. The structure of $FADH_2$ is presented in Figure 2–83B. (A, courtesy of Daniel S. Friend.)

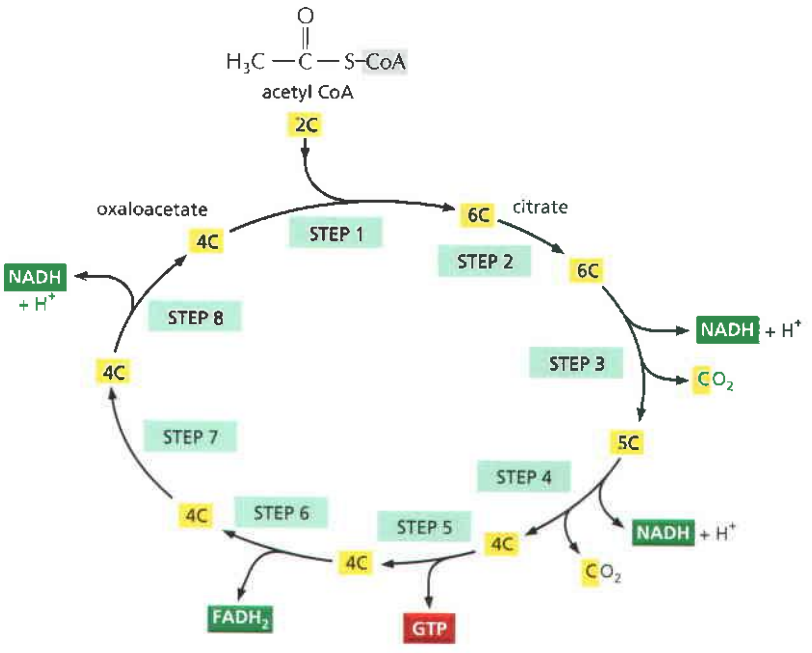


eventually focused on the oxidation of pyruvate and led in 1937 to the discovery of the **citric acid cycle**, also known as the *tricarboxylic acid cycle* or the *Krebs cycle*. The citric acid cycle accounts for about two-thirds of the total oxidation of carbon compounds in most cells, and its major end products are CO_2 and high-energy electrons in the form of NADH. The CO_2 is released as a waste product, while the high-energy electrons from NADH are passed to a membrane-bound electron-transport chain (discussed in Chapter 14), eventually combining with O_2 to produce H_2O. Although the citric acid cycle itself does not use O_2, it requires O_2 in order to proceed because there is no other efficient way for the NADH to get rid of its electrons and thus regenerate the NAD^+ that is needed to keep the cycle going.

The citric acid cycle takes place inside mitochondria in eucaryotic cells. It results in the complete oxidation of the carbon atoms of the acetyl groups in acetyl CoA, converting them into CO_2. But the acetyl group is not oxidized directly. Instead, this group is transferred from acetyl CoA to a larger, four-carbon molecule, *oxaloacetate*, to form the six-carbon tricarboxylic acid, *citric acid*, for which the subsequent cycle of reactions is named. The citric acid molecule is then gradually oxidized, allowing the energy of this oxidation to be harnessed to produce energy-rich activated carrier molecules. The chain of eight reactions forms a cycle because at the end the oxaloacetate is regenerated and enters a new turn of the cycle, as shown in outline in **Figure 2–82**.

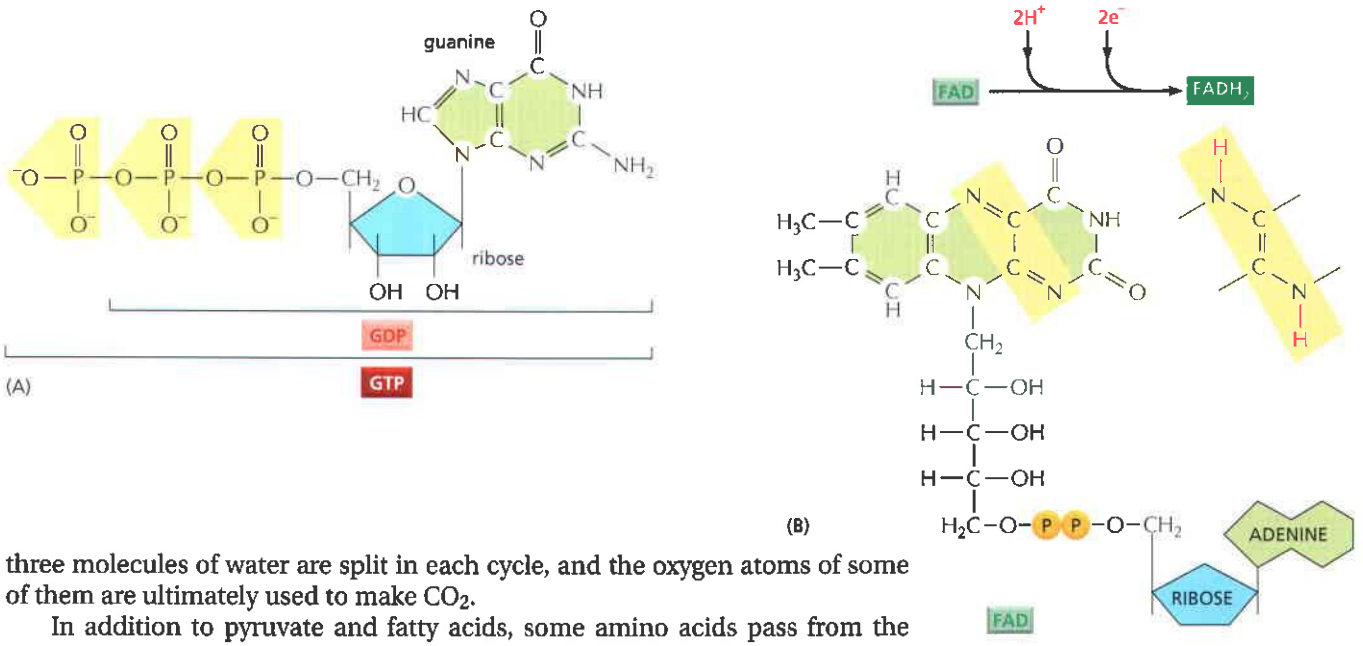
We have thus far discussed only one of the three types of activated carrier molecules that are produced by the citric acid cycle, the NAD^+–NADH pair (see Figure 2–60). In addition to three molecules of NADH, each turn of the cycle also produces one molecule of **$FADH_2$** (reduced flavin adenine dinucleotide) from FAD and one molecule of the ribonucleotide **GTP** (guanosine triphosphate) from GDP. The structures of these two activated carrier molecules are illustrated in **Figure 2–83**. GTP is a close relative of ATP, and the transfer of its terminal phosphate group to ADP produces one ATP molecule in each cycle. Like NADH, $FADH_2$ is a carrier of high-energy electrons and hydrogen. As we discuss shortly, the energy that is stored in the readily transferred high-energy electrons of NADH and $FADH_2$ will be utilized subsequently for ATP production through the process of *oxidative phosphorylation*, the only step in the oxidative catabolism of foodstuffs that directly requires gaseous oxygen (O_2) from the atmosphere.

Panel 2–9 (pp. 122–123) presents the complete citric acid cycle. Water, rather than molecular oxygen, supplies the extra oxygen atoms required to make CO_2 from the acetyl groups entering the citric acid cycle. As illustrated in the panel,



NET RESULT: ONE TURN OF THE CYCLE PRODUCES THREE NADH, ONE GTP, AND ONE $FADH_2$, AND RELEASES TWO MOLECULES OF CO_2

Figure 2–82 Simple overview of the citric acid cycle. <TAGT> The reaction of acetyl CoA with oxaloacetate starts the cycle by producing citrate (citric acid). In each turn of the cycle, two molecules of CO_2 are produced as waste products, plus three molecules of NADH, one molecule of GTP, and one molecule of $FADH_2$. The number of carbon atoms in each intermediate is shown in a *yellow box*. For details, see Panel 2–9 (pp. 122–123).



three molecules of water are split in each cycle, and the oxygen atoms of some of them are ultimately used to make CO_2.

In addition to pyruvate and fatty acids, some amino acids pass from the cytosol into mitochondria, where they are also converted into acetyl CoA or one of the other intermediates of the citric acid cycle. Thus, in the eucaryotic cell, the mitochondrion is the center toward which all energy-yielding processes lead, whether they begin with sugars, fats, or proteins.

Both the citric acid cycle and glycolysis also function as starting points for important biosynthetic reactions by producing vital carbon-containing intermediates, such as *oxaloacetate* and *α-ketoglutarate*. Some of these substances produced by catabolism are transferred back from the mitochondrion to the cytosol, where they serve in anabolic reactions as precursors for the synthesis of many essential molecules, such as amino acids (**Figure 2–84**).

Figure 2–83 The structures of GTP and $FADH_2$. (A) GTP and GDP are close relatives of ATP and ADP, respectively. (B) $FADH_2$ is a carrier of hydrogens and high-energy electrons, like NADH and NADPH. It is shown here in its oxidized form (FAD) with the hydrogen-carrying atoms highlighted in *yellow*.

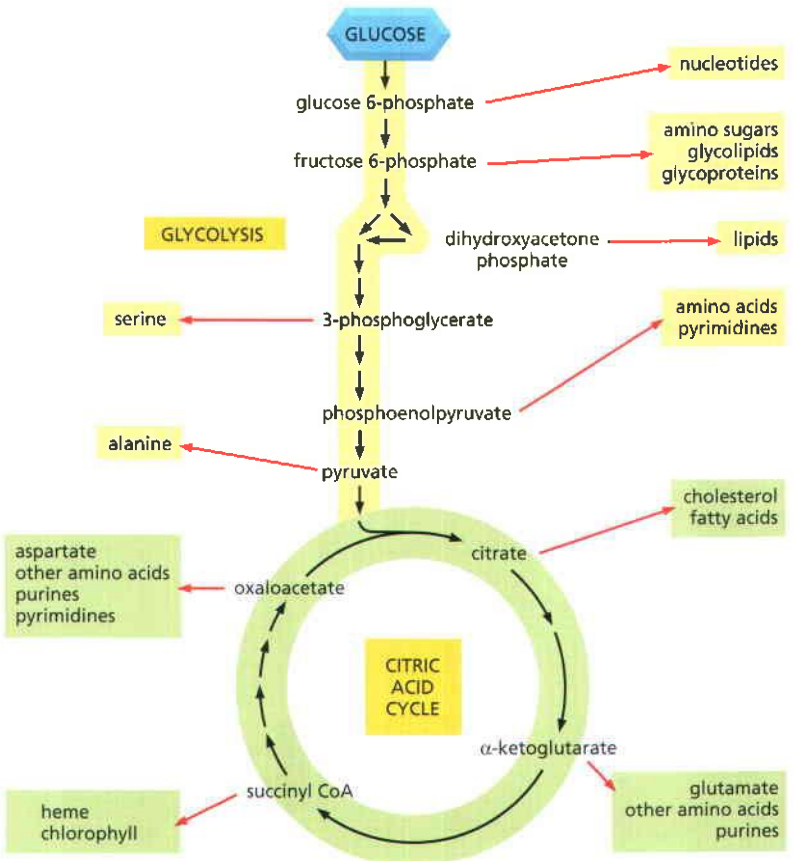


Figure 2–84 Glycolysis and the citric acid cycle provide the precursors needed to synthesize many important biological molecules. The amino acids, nucleotides, lipids, sugars, and other molecules—shown here as products—in turn serve as the precursors for the many macromolecules of the cell. Each *black arrow* in this diagram denotes a single enzyme-catalyzed reaction; the *red arrows* generally represent pathways with many steps that are required to produce the indicated products.

Electron Transport Drives the Synthesis of the Majority of the ATP in Most Cells

Most chemical energy is released in the last step in the degradation of a food molecule. In this final process the electron carriers NADH and $FADH_2$ transfer the electrons that they have gained when oxidizing other molecules to the **electron-transport chain**, which is embedded in the inner membrane of the mitochondrion (see Figure 14–10). As the electrons pass along this long chain of specialized electron acceptor and donor molecules, they fall to successively lower energy states. The energy that the electrons release in this process pumps H^+ ions (protons) across the membrane—from the inner mitochondrial compartment to the outside—generating a gradient of H^+ ions (Figure 2–85). This gradient serves as a source of energy, being tapped like a battery to drive a variety of energy-requiring reactions. The most prominent of these reactions is the generation of ATP by the phosphorylation of ADP.

At the end of this series of electron transfers, the electrons are passed to molecules of oxygen gas (O_2) that have diffused into the mitochondrion, which simultaneously combine with protons (H^+) from the surrounding solution to produce water molecules. The electrons have now reached their lowest energy level, and therefore all the available energy has been extracted from the oxidized food molecule. This process, termed **oxidative phosphorylation** (Figure 2–86), also occurs in the plasma membrane of bacteria. As one of the most remarkable achievements of cell evolution, it is a central topic of Chapter 14.

In total, the complete oxidation of a molecule of glucose to H_2O and CO_2 is used by the cell to produce about 30 molecules of ATP. In contrast, only 2 molecules of ATP are produced per molecule of glucose by glycolysis alone.

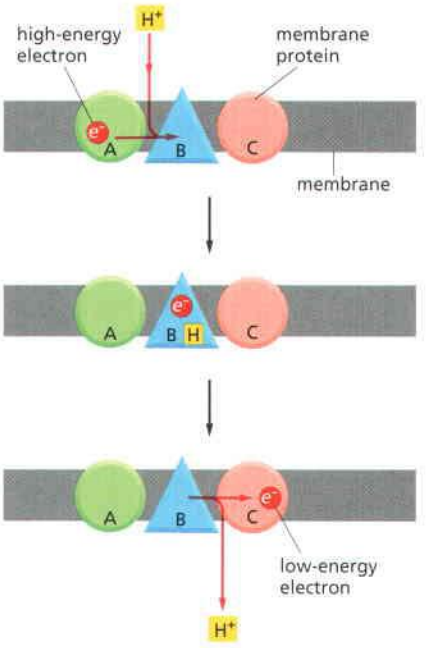


Figure 2–85 The generation of an H^+ gradient across a membrane by electron-transport reactions. A high-energy electron (derived, for example, from the oxidation of a metabolite) is passed sequentially by carriers A, B, and C to a lower energy state. In this diagram carrier B is arranged in the membrane in such a way that it takes up H^+ from one side and releases it to the other as the electron passes. The result is an H^+ gradient. As discussed in Chapter 14, this gradient is an important form of energy that is harnessed by other membrane proteins to drive the formation of ATP.

Amino Acids and Nucleotides Are Part of the Nitrogen Cycle

So far we have concentrated mainly on carbohydrate metabolism and have not yet considered the metabolism of nitrogen or sulfur. These two elements are important constituents of biological macromolecules. Nitrogen and sulfur atoms pass from compound to compound and between organisms and their environment in a series of reversible cycles.

Although molecular nitrogen is abundant in the Earth's atmosphere, nitrogen is chemically unreactive as a gas. Only a few living species are able to incorporate it into organic molecules, a process called **nitrogen fixation**. Nitrogen fixation occurs in certain microorganisms and by some geophysical processes, such as lightning discharge. It is essential to the biosphere as a whole, for without it life could not exist on this planet. Only a small fraction of the nitrogenous compounds in today's organisms, however, is due to fresh products of nitrogen fixation from the atmosphere. Most organic nitrogen has been in circulation for

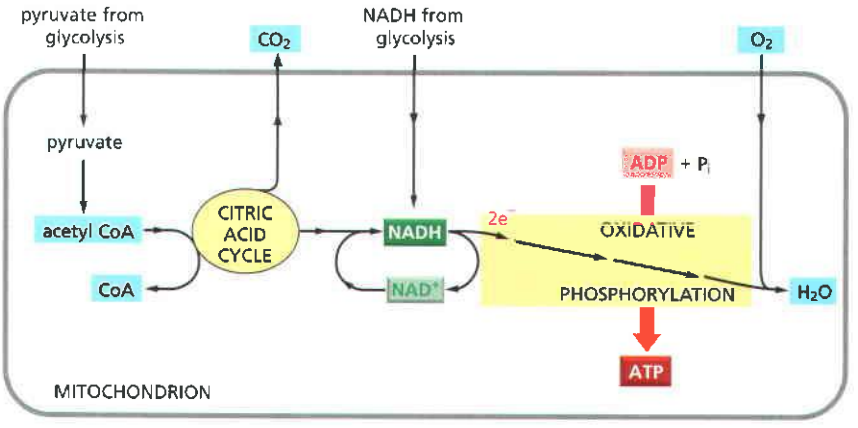


Figure 2–86 The final stages of oxidation of food molecules. Molecules of NADH and $FADH_2$ ($FADH_2$ is not shown) are produced by the citric acid cycle. These activated carriers donate high-energy electrons that are eventually used to reduce oxygen gas to water. A major portion of the energy released during the transfer of these electrons along an electron-transfer chain in the mitochondrial inner membrane (or in the plasma membrane of bacteria) is harnessed to drive the synthesis of ATP—hence the name oxidative phosphorylation (discussed in Chapter 14).

some time, passing from one living organism to another. Thus present-day nitrogen-fixing reactions can be said to perform a "topping-up" function for the total nitrogen supply.

Vertebrates receive virtually all of their nitrogen from their dietary intake of proteins and nucleic acids. In the body these macromolecules are broken down to amino acids and the components of nucleotides, and the nitrogen they contain is used to produce new proteins and nucleic acids—or utilized to make other molecules. About half of the 20 amino acids found in proteins are essential amino acids for vertebrates (**Figure 2–87**), which means that they cannot be synthesized from other ingredients of the diet. The others can be so synthesized, using a variety of raw materials, including intermediates of the citric acid cycle as described previously. The essential amino acids are made by plants and other organisms, usually by long and energetically expensive pathways that have been lost in the course of vertebrate evolution. RoshanKetab 021-66950639

The nucleotides needed to make RNA and DNA can be synthesized using specialized biosynthetic pathways. All of the nitrogens in the purine and pyrimidine bases (as well as some of the carbons) are derived from the plentiful amino acids glutamine, aspartic acid, and glycine, whereas the ribose and deoxyribose sugars are derived from glucose. There are no "essential nucleotides" that must be provided in the diet.

Amino acids not used in biosynthesis can be oxidized to generate metabolic energy. Most of their carbon and hydrogen atoms eventually form CO_2 or H_2O, whereas their nitrogen atoms are shuttled through various forms and eventually appear as urea, which is excreted. Each amino acid is processed differently, and a whole constellation of enzymatic reactions exists for their catabolism.

Sulfur is abundant on Earth in its most oxidized form, sulfate (SO_4^{2-}). To convert it to forms useful for life, sulfate must be reduced to sulfide (S^{2-}), the oxidation state of sulfur required for the synthesis of essential biological molecules. These molecules include the amino acids methionine and cysteine, coenzyme A (see Figure 2–62), and the iron-sulfur centers essential for electron transport (see Figure 14–23). The process begins in bacteria, fungi, and plants, where a special group of enzymes use ATP and reducing power to create a sulfate assimilation pathway. Humans and other animals cannot reduce sulfate and must therefore acquire the sulfur they need for their metabolism in the food that they eat.



Figure 2–87 The nine essential amino acids. These cannot be synthesized by human cells and so must be supplied in the diet.

Metabolism Is Organized and Regulated

One gets a sense of the intricacy of a cell as a chemical machine from the relation of glycolysis and the citric acid cycle to the other metabolic pathways sketched out in **Figure 2–88**. This type of chart, which was used earlier in this chapter to introduce metabolism, represents only some of the enzymatic pathways in a cell. It is obvious that our discussion of cell metabolism has dealt with only a tiny fraction of cellular chemistry.

All these reactions occur in a cell that is less than 0.1 mm in diameter, and each requires a different enzyme. As is clear from Figure 2–88, the same molecule can often be part of many different pathways. Pyruvate, for example, is a substrate for half a dozen or more different enzymes, each of which modifies it chemically in a different way. One enzyme converts pyruvate to acetyl CoA, another to oxaloacetate; a third enzyme changes pyruvate to the amino acid alanine, a fourth to lactate, and so on. All of these different pathways compete for the same pyruvate molecule, and similar competitions for thousands of other small molecules go on at the same time.

The situation is further complicated in a multicellular organism. Different cell types will in general require somewhat different sets of enzymes. And different tissues make distinct contributions to the chemistry of the organism as a whole. In addition to differences in specialized products such as hormones or antibodies, there are significant differences in the "common" metabolic pathways among various types of cells in the same organism.

Although virtually all cells contain the enzymes of glycolysis, the citric acid cycle, lipid synthesis and breakdown, and amino acid metabolism, the levels of

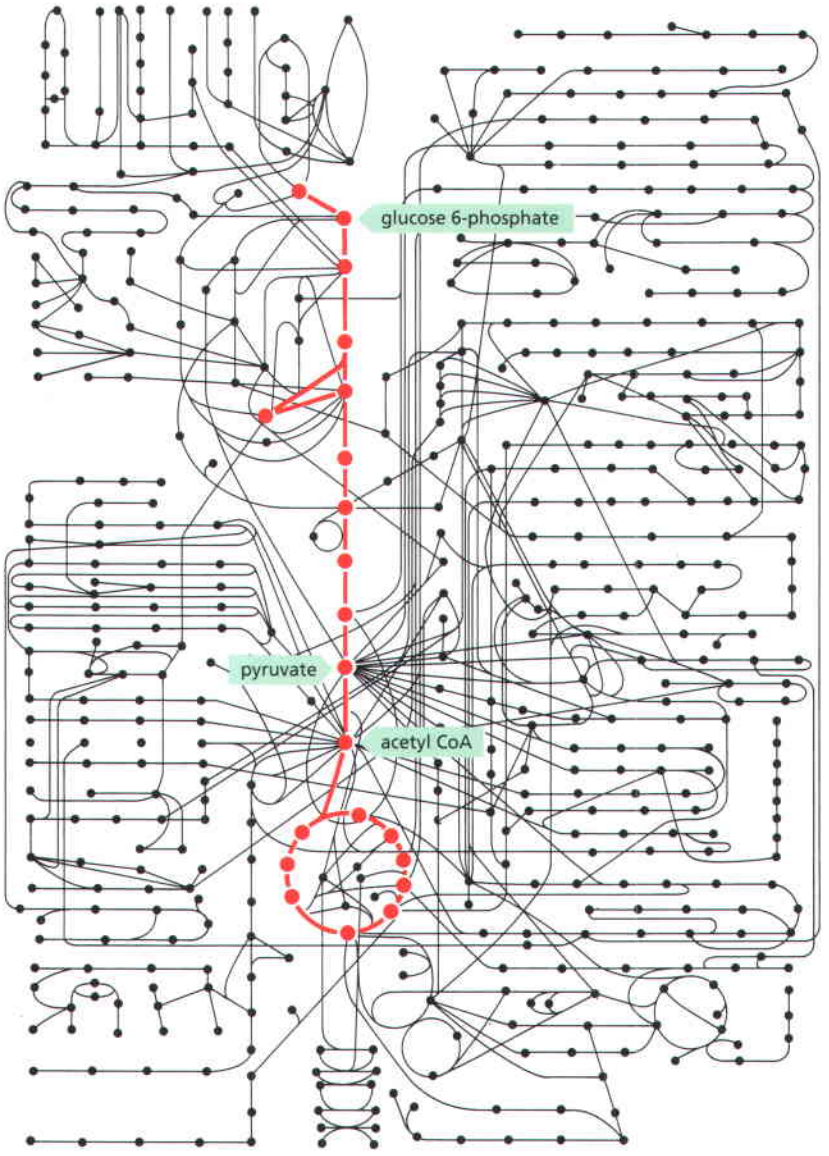


Figure 2–88 Glycolysis and the citric acid cycle are at the center of metabolism. Some 500 metabolic reactions of a typical cell are shown schematically with the reactions of glycolysis and the citric acid cycle in *red*. Other reactions either lead into these two central pathways—delivering small molecules to be catabolized with production of energy—or they lead outward and thereby supply carbon compounds for the purpose of biosynthesis.

these processes required in different tissues are not the same. For example, nerve cells, which are probably the most fastidious cells in the body, maintain almost no reserves of glycogen or fatty acids and rely almost entirely on a constant supply of glucose from the bloodstream. In contrast, liver cells supply glucose to actively contracting muscle cells and recycle the lactic acid produced by muscle cells back into glucose. All types of cells have their distinctive metabolic traits, and they cooperate extensively in the normal state, as well as in response to stress and starvation. One might think that the whole system would need to be so finely balanced that any minor upset, such as a temporary change in dietary intake, would be disastrous.

In fact, the metabolic balance of a cell is amazingly stable. Whenever the balance is perturbed, the cell reacts so as to restore the initial state. The cell can adapt and continue to function during starvation or disease. Mutations of many kinds can damage or even eliminate particular reaction pathways, and yet—provided that certain minimum requirements are met—the cell survives. It does so because an elaborate network of *control mechanisms* regulates and coordinates the rates of all of its reactions. These controls rest, ultimately, on the remarkable abilities of proteins to change their shape and their chemistry in response to changes in their immediate environment. The principles that underlie how large molecules such as proteins are built and the chemistry behind their regulation will be our next concern.

Summary

Glucose and other food molecules are broken down by controlled stepwise oxidation to provide chemical energy in the form of ATP and NADH. There are three main sets of reactions that act in series—the products of each being the starting material for the next: glycolysis (which occurs in the cytosol), the citric acid cycle (in the mitochondrial matrix), and oxidative phosphorylation (on the inner mitochondrial membrane). The intermediate products of glycolysis and the citric acid cycle are used both as sources of metabolic energy and to produce many of the small molecules used as the raw materials for biosynthesis. Cells store sugar molecules as glycogen in animals and starch in plants; both plants and animals also use fats extensively as a food store. These storage materials in turn serve as a major source of food for humans, along with the proteins that comprise the majority of the dry mass of most of the cells in the foods we eat.

PROBLEMS

Which statements are true? Explain why or why not.

2-1 Of the original radioactivity in a sample, only about 1/1000 will remain after 10 half-lives.

2-2 A 10^{-8} M solution of HCl has a pH of 8.

2-3 Most of the interactions between macromolecules could be mediated just as well by covalent bonds as by noncovalent bonds.

2-4 Animals and plants use oxidation to extract energy from food molecules.

2-5 If an oxidation occurs in a reaction, it must be accompanied by a reduction.

2-6 Linking the energetically unfavorable reaction A → B to a second, favorable reaction B → C will shift the equilibrium constant for the first reaction.

2-7 The criterion for whether a reaction proceeds spontaneously is ΔG not $\Delta G°$, because ΔG takes into account the concentrations of the substrates and products.

2-8 Because glycolysis is only a prelude to the oxidation of glucose in mitochondria, which yields 15-fold more ATP, glycolysis is not really important for human cells.

2-9 The oxygen consumed during the oxidation of glucose in animal cells is returned as CO_2 to the atmosphere.

Discuss the following problems.

2-10 The organic chemistry of living cells is said to be special for two reasons: it occurs in an aqueous environment and it accomplishes some very complex reactions. But do you suppose it is really all that much different from the organic chemistry carried out in the top laboratories in the world? Why or why not?

2-11 The molecular weight of ethanol (CH_3CH_2OH) is 46 and its density is 0.789 g/cm^3.

A. What is the molarity of ethanol in beer that is 5% ethanol by volume? [Alcohol content of beer varies from about 4% (lite beer) to 8% (stout beer).]

B. The legal limit for a driver's blood alcohol content varies, but 80 mg of ethanol per 100 mL of blood (usually referred to as a blood alcohol level of 0.08) is typical. What is the molarity of ethanol in a person at this legal limit?

C. How many 12-oz (355-mL) bottles of 5% beer could a 70-kg person drink and remain under the legal limit? A 70-kg person contains about 40 liters of water. Ignore the metabolism of ethanol, and assume that the water content of the person remains constant.

D. Ethanol is metabolized at a constant rate of about 120 mg per hour per kg body weight, regardless of its concentration. If a 70-kg person were at twice the legal limit (160 mg/100 mL), how long would it take for their blood alcohol level to fall below the legal limit?

Table Q2–1 Radioactive isotopes and some of their properties (Problem 2–12).

RADIOACTIVE ISOTOPE	EMISSION	HALF-LIFE	MAXIMUM SPECIFIC ACTIVITY (Ci/mmol)
^{14}C	β particle	5730 years	0.062
^{3}H	β particle	12.3 years	29
^{35}S	β particle	87.4 days	1490
^{32}P	β particle	14.3 days	9120

2-12 Specific activity refers to the amount of radioactivity per unit amount of substance, usually in biology expressed on a molar basis, for example, as Ci/mmol. [One curie (Ci) corresponds to 2.22×10^{12} disintegrations per minute (dpm).] As apparent in **Table Q2–1**, which lists properties of four isotopes commonly used in biology, there is an inverse relationship between maximum specific activity and half-life. Do you suppose this is just a coincidence or is there an underlying reason? Explain your answer.

2-13 By a convenient coincidence the ion product of water, $K_W = [H^+][OH^-]$, is a nice round number: 1.0×10^{-14} M^2.

A. Why is a solution at pH 7.0 said to be neutral?

B. What is the H^+ concentration and pH of a 1 mM solution of NaOH?

C. If the pH of a solution is 5.0, what is the concentration of OH^- ions?

2-14 Suggest a rank order for the pK values (from lowest to highest) for the carboxyl group on the aspartate side chain

in the following environments in a protein. Explain your ranking.

1. An aspartate side chain on the surface of a protein with no other ionizable groups nearby.
2. An aspartate side chain buried in a hydrophobic pocket on the surface of a protein.
3. An aspartate side chain in a hydrophobic pocket adjacent to a glutamate side chain.
4. An aspartate side chain in a hydrophobic pocket adjacent to a lysine side chain.

2–15 A histidine side chain is known to play an important role in the catalytic mechanism of an enzyme; however, it is not clear whether histidine is required in its protonated (charged) or unprotonated (uncharged) state. To answer this question you measure enzyme activity over a range of pH, with the results shown in **Figure Q2–1**. Which form of histidine is required for enzyme activity?

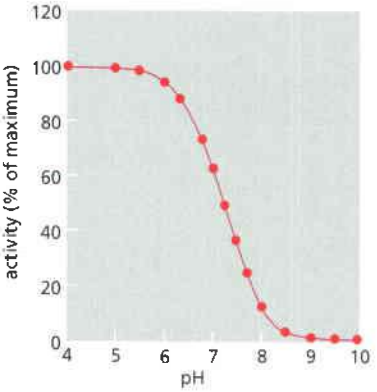


Figure Q2–1 Enzyme activity as a function of pH (Problem 2–15).

2–16 During an all-out sprint, muscles metabolize glucose anaerobically, producing a high concentration of lactic acid, which lowers the pH of the blood and of the cytosol and contributes to the fatigue sprinters experience well before their fuel reserves are exhausted. The main blood buffer against pH changes is the bicarbonate/CO_2 system.

$$\underset{\text{(gas)}}{CO_2} \rightleftharpoons \underset{\text{(dissolved)}}{CO_2} \overset{\text{p}K_1 = 2.3}{\rightleftharpoons} H_2CO_3 \overset{\text{p}K_2 = 3.8}{\rightleftharpoons} H^+ + HCO_3^- \overset{\text{p}K_3 = 10.3}{\rightleftharpoons} H^+ + CO_3^{2-}$$

To improve their performance, would you advise sprinters to hold their breath or to breathe rapidly for a minute immediately before the race? Explain your answer.

2–17 The three molecules in **Figure Q2–2** contain the seven most common reactive groups in biology. Most molecules in the cell are built from these functional groups. Indicate and name the functional groups in these molecules.

2–18 "Diffusion" sounds slow—and over everyday distances it is—but on the scale of a cell it is very fast. The average instantaneous velocity of a particle in solution, that is, the velocity between collisions, is

$$v = (kT/m)^{1/2}$$

where $k = 1.38 \times 10^{-16}$ g cm^2/K sec^2, T = temperature in K (37°C is 310 K), m = mass in g/molecule.

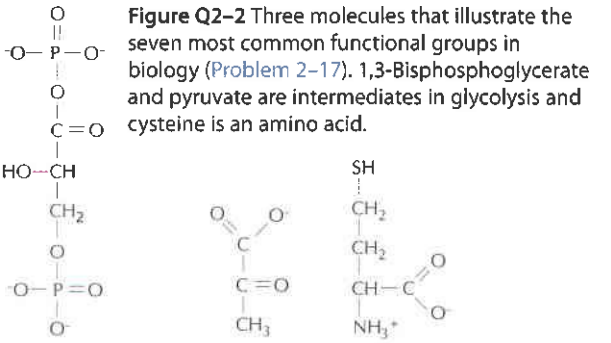


Figure Q2–2 Three molecules that illustrate the seven most common functional groups in biology (Problem 2–17). 1,3-Bisphosphoglycerate and pyruvate are intermediates in glycolysis and cysteine is an amino acid.

Calculate the instantaneous velocity of a water molecule (molecular mass = 18 daltons), a glucose molecule (molecular mass = 180 daltons), and a myoglobin molecule (molecular mass = 15,000 daltons) at 37°C. Just for fun, convert these numbers into kilometers/hour. Before you do any calculations, try to guess whether the molecules are moving at a slow crawl (<1 km/hr), an easy walk (5 km/hr), or a record-setting sprint (40 km/hr).

2–19 Polymerization of tubulin subunits into microtubules occurs with an increase in the orderliness of the subunits (**Figure Q2–3**). Yet tubulin polymerization occurs with an increase in entropy (decrease in order). How can that be?

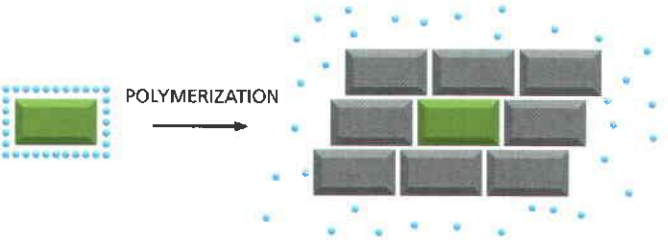


Figure Q2–3 Polymerization of tubulin subunits into a microtubule (Problem 2–19). The fates of one subunit (*shaded*) and its associated water molecules (*small spheres*) are shown.

2–20 A 70-kg adult human (154 lb) could meet his or her entire energy needs for one day by eating 3 moles of glucose (540 g). (We don't recommend this.) Each molecule of glucose generates 30 ATP when it is oxidized to CO_2. The concentration of ATP is maintained in cells at about 2 mM, and a 70-kg adult has about 25 liters of intracellular fluid. Given that the ATP concentration remains constant in cells, calculate how many times per day, on average, each ATP molecule in the body is hydrolyzed and resynthesized.

2–21 Assuming that there are 5×10^{13} cells in the human body and that ATP is turning over at a rate of 10^9 ATP per minute in each cell, how many watts is the human body consuming? (A watt is a joule per second, and there are 4.18 joules/calorie.) Assume that hydrolysis of ATP yields 12 kcal/mole.

2–22 Does a Snickers™ candy bar (65 g, 325 kcal) provide enough energy to climb from Zermatt (elevation 1660 m) to the top of the Matterhorn (4478 m, **Figure Q2–4**), or might



Figure Q2–4 The Matterhorn (Problem 2–22). (Courtesy of Zermatt Tourism.)

you need to stop at Hörnli Hut (3260 m) to eat another one? Imagine that you and your gear have a mass of 75 kg, and that all of your work is done against gravity (that is, you are just climbing straight up). Remember from your introductory physics course that

$$\text{work (J)} = \text{mass (kg)} \times g\,(\text{m/sec}^2) \times \text{height gained (m)}$$

where g is acceleration due to gravity (9.8 m/sec^2). One joule is 1 kg m^2/sec^2 and there are 4.18 kJ per kcal.

What assumptions made here will greatly underestimate how much candy you need?

2–23 At first glance, fermentation of pyruvate to lactate appears to be an optional add-on reaction to glycolysis. After all, could cells growing in the absence of oxygen not simply discard pyruvate as a waste product? In the absence of fermentation, which products derived from glycolysis would accumulate in cells under anaerobic conditions? Could the metabolism of glucose via the glycolytic pathway continue in the absence of oxygen in cells that cannot carry out fermentation? Why or why not?

2–24 In the absence of oxygen, cells consume glucose at a high, steady rate. When oxygen is added, glucose consumption drops precipitously and is then maintained at the lower rate. Why is glucose consumed at a high rate in the absence of oxygen and at a low rate in its presence?

2–25 The liver provides glucose to the rest of the body between meals. It does so by breaking down glycogen, forming glucose 6-phosphate in the penultimate step. Glucose 6-phosphate is converted to glucose by splitting off the phosphate (ΔG° = –3.3 kcal/mole). Why do you suppose the liver removes the phosphate by hydrolysis, rather than reversing the reaction by which glucose 6-phosphate (G6P) is formed from glucose (glucose + ATP → G6P + ADP, ΔG° = –4.0 kcal/mole)? By reversing this reaction the liver could generate both glucose and ATP.

2–26 In 1904 Franz Knoop performed what was probably the first successful labeling experiment to study metabolic pathways. He fed many different fatty acids labeled with a terminal benzene ring to dogs and analyzed their urine for excreted benzene derivatives. Whenever the fatty acid had an even number of carbon atoms, phenylacetate was excreted (**Figure Q2–5**A). Whenever the fatty acid had an odd number of carbon atoms, benzoate was excreted (Figure Q2–5B).

From these experiments Knoop deduced that oxidation of fatty acids to CO_2 and H_2O involved the removal of two-carbon fragments from the carboxylic acid end of the chain.

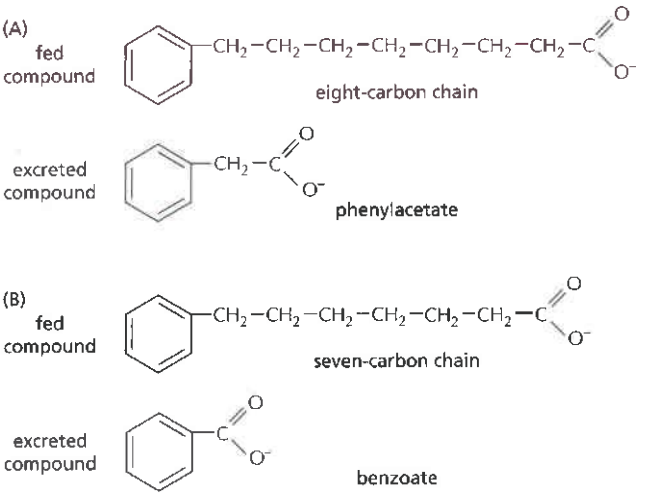


Figure Q2–5 The original labeling experiment to analyze fatty acid oxidation (Problem 2–26). (A) Fed and excreted derivatives of an even-number fatty acid chain. (B) Fed and excreted derivatives of an odd-number fatty acid chain.

Can you explain the reasoning that led him to conclude that two-carbon fragments, as opposed to any other number, were removed, and that degradation was from the carboxylic acid end, as opposed to the other end?

2–27 Pathways for synthesis of amino acids in microorganisms were worked out in part by cross-feeding experiments among mutant organisms that were defective for individual steps in the pathway. Results of cross-feeding experiments for three mutants defective in the tryptophan pathway—*TrpB*⁻, *TrpD*⁻, and *TrpE*⁻—are shown in **Figure Q2–6**. The mutants were streaked on a Petri dish and allowed to grow briefly in the presence of a very small amount of tryptophan, producing three pale streaks. As shown, heavier growth was observed at points where some streaks were close to other streaks. These spots of heavier growth indicate that one mutant can cross-feed (supply an intermediate) to the other one.

From the pattern of cross-feeding shown in Figure Q2–6, deduce the order of the steps controlled by the products of the *TrpB*, *TrpD*, and *TrpE* genes. Explain your reasoning.

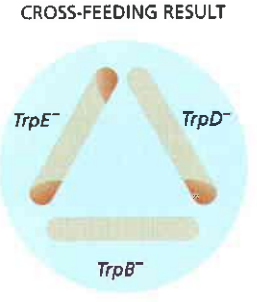
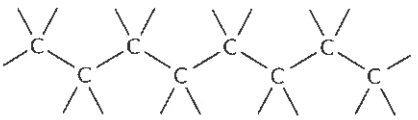


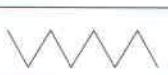
Figure Q2–6 Defining the pathway for tryptophan synthesis using cross-feeding experiments (Problem 2–27). Results of a cross-feeding experiment among mutants defective for steps in the tryptophan biosynthetic pathway. *Dark areas* on the Petri dish show regions of cell growth.

CARBON SKELETONS

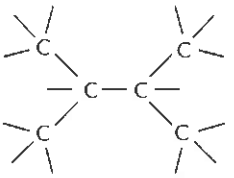
Carbon has a unique role in the cell because of its ability to form strong covalent bonds with other carbon atoms. Thus carbon atoms can join to form chains.



also written as



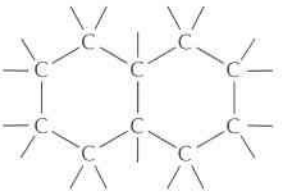
or branched trees



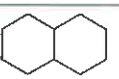
also written as



or rings



also written as



COVALENT BONDS

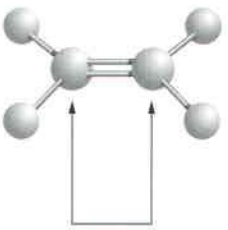
A covalent bond forms when two atoms come very close together and share one or more of their electrons. In a single bond one electron from each of the two atoms is shared; in a double bond a total of four electrons are shared.

Each atom forms a fixed number of covalent bonds in a defined spatial arrangement. For example, carbon forms four single bonds arranged tetrahedrally, whereas nitrogen forms three single bonds and oxygen forms two single bonds arranged as shown below.



Double bonds exist and have a different spatial arrangement.





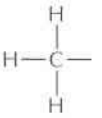
Atoms joined by two or more covalent bonds cannot rotate freely around the bond axis. This restriction is a major influence on the three-dimensional shape of many macromolecules.

HYDROCARBONS

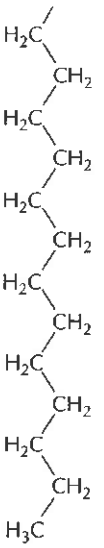
Carbon and hydrogen combine together to make stable compounds (or chemical groups) called hydrocarbons. These are nonpolar, do not form hydrogen bonds, and are generally insoluble in water.



methane



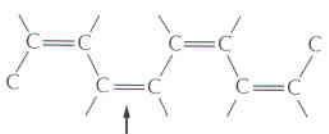
methyl group



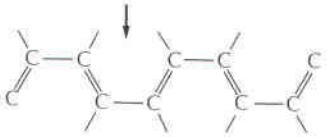
part of the hydrocarbon "tail" of a fatty acid molecule

ALTERNATING DOUBLE BONDS

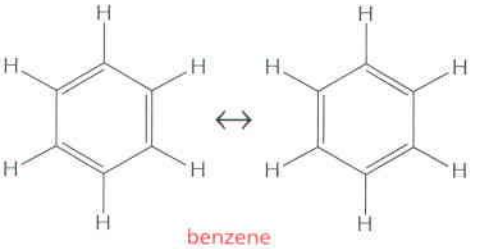
The carbon chain can include double bonds. If these are on alternate carbon atoms, the bonding electrons move within the molecule, stabilizing the structure by a phenomenon called resonance.



the truth is somewhere between these two structures



Alternating double bonds in a ring can generate a very stable structure.



benzene

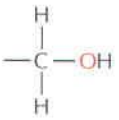
often written as



C–O CHEMICAL GROUPS

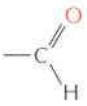
Many biological compounds contain a carbon bonded to an oxygen. For example,

alcohol

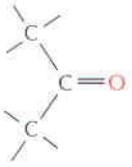


The –OH is called a hydroxyl group.

aldehyde

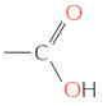


ketone



The C=O is called a carbonyl group.

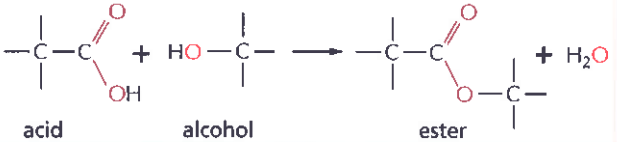
carboxylic acid



The –COOH is called a carboxyl group. In water this loses an H^+ ion to become $–COO^-$.

esters

Esters are formed by a condensation reaction between acid and an alcohol.

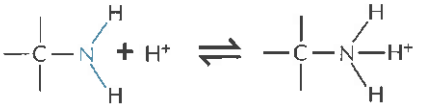


acid alcohol ester

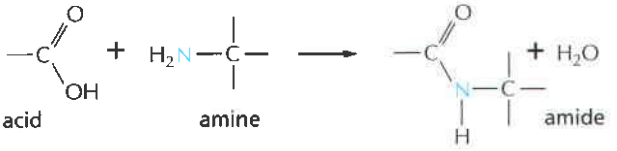
C–N CHEMICAL GROUPS

Amines and amides are two important examples of compounds containing a carbon linked to a nitrogen.

Amines in water combine with an H^+ ion to become positively charged.

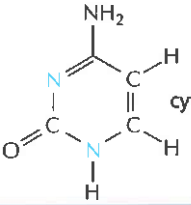


Amides are formed by combining an acid and an amine. Unlike amines, amides are uncharged in water. An example is the peptide bond that joins amino acids in a protein.



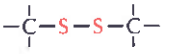
acid amine amide

Nitrogen also occurs in several ring compounds, including important constituents of nucleic acids: purines and pyrimidines.



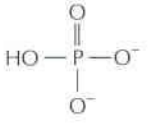
cytosine (a pyrimidine)

SULFHYDRYL GROUP

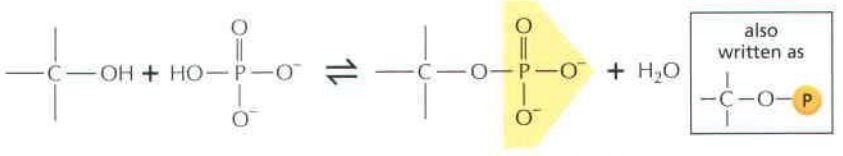
The  is called a sulfhydryl group. In the amino acid cysteine the sulfhydryl group may exist in the reduced form, 
or more rarely in an oxidized, cross-bridging form, 

PHOSPHATES

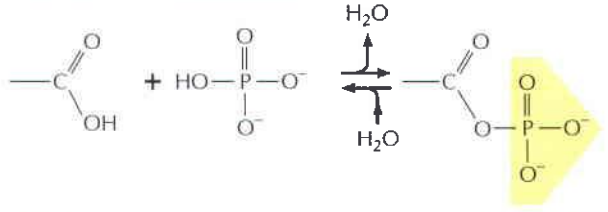
Inorganic phosphate is a stable ion formed from phosphoric acid, H_3PO_4. It is often written as P_i.



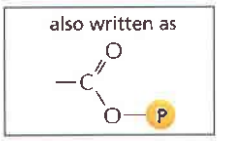
Phosphate esters can form between a phosphate and a free hydroxyl group. Phosphate groups are often attached to proteins in this way.

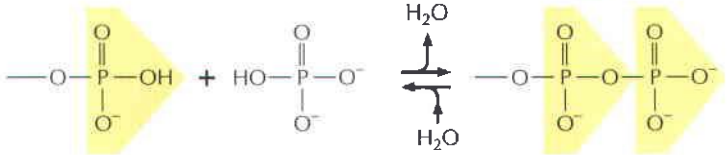


The combination of a phosphate and a carboxyl group, or two or more phosphate groups, gives an acid anhydride.

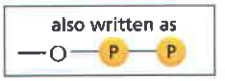


high-energy acyl phosphate bond (carboxylic–phosphoric acid anhydride) found in some metabolites



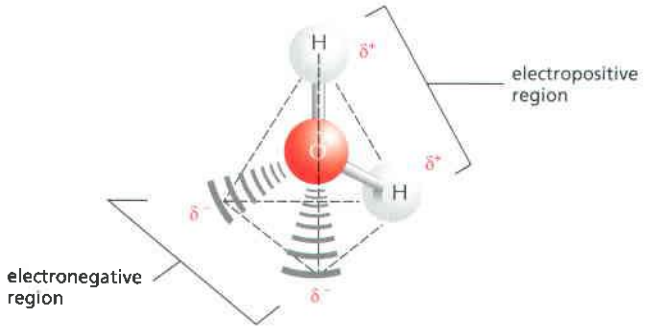


phosphoanhydride—a high-energy bond found in molecules such as ATP



WATER

Two atoms, connected by a covalent bond, may exert different attractions for the electrons of the bond. In such cases the bond is polar, with one end slightly negatively charged (δ^-) and the other slightly positively charged (δ^+).



Although a water molecule has an overall neutral charge (having the same number of electrons and protons), the electrons are asymmetrically distributed, which makes the molecule polar. The oxygen nucleus draws electrons away from the hydrogen nuclei, leaving these nuclei with a small net positive charge. The excess of electron density on the oxygen atom creates weakly negative regions at the other two corners of an imaginary tetrahedron.

WATER STRUCTURE

Molecules of water join together transiently in a hydrogen-bonded lattice. Even at 37°C, 15% of the water molecules are joined to four others in a short-lived assembly known as a "flickering cluster."

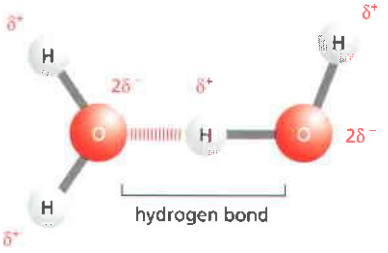


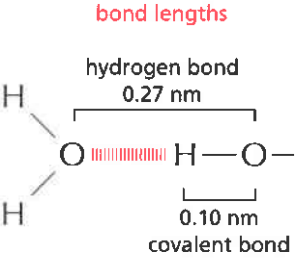
The cohesive nature of water is responsible for many of its unusual properties, such as high surface tension, specific heat, and heat of vaporization.

HYDROGEN BONDS

Because they are polarized, two adjacent H_2O molecules can form a linkage known as a hydrogen bond. Hydrogen bonds have only about 1/20 the strength of a covalent bond.

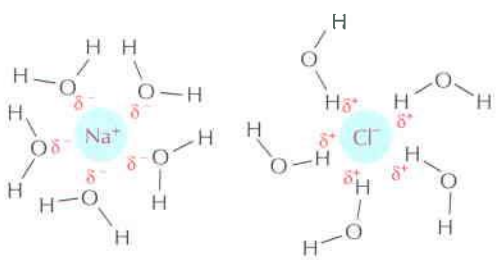
Hydrogen bonds are strongest when the three atoms lie in a straight line.





HYDROPHILIC MOLECULES

Substances that dissolve readily in water are termed hydrophilic. They are composed of ions or polar molecules that attract water molecules through electrical charge effects. Water molecules surround each ion or polar molecule on the surface of a solid substance and carry it into solution.



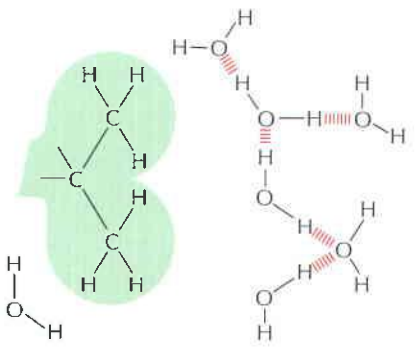
Ionic substances such as sodium chloride dissolve because water molecules are attracted to the positive (Na^+) or negative (Cl^-) charge of each ion.



Polar substances such as urea dissolve because their molecules form hydrogen bonds with the surrounding water molecules.

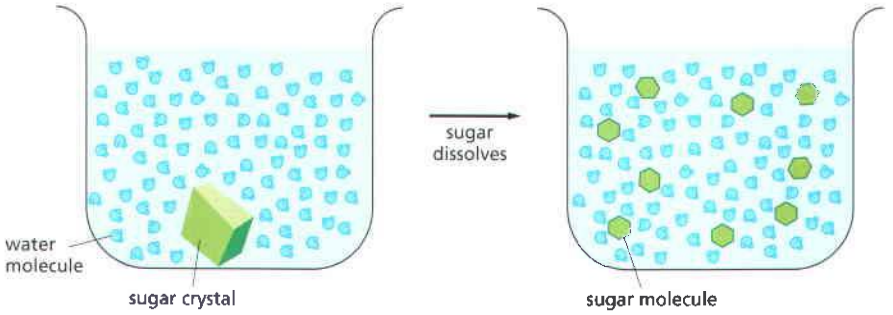
HYDROPHOBIC MOLECULES

Molecules that contain a preponderance of nonpolar bonds are usually insoluble in water and are termed hydrophobic. This is true, especially, of hydrocarbons, which contain many C–H bonds. Water molecules are not attracted to such molecules and so have little tendency to surround them and carry them into solution.



WATER AS A SOLVENT

Many substances, such as household sugar, dissolve in water. That is, their molecules separate from each other, each becoming surrounded by water molecules.



When a substance dissolves in a liquid, the mixture is termed a solution. The dissolved substance (in this case sugar) is the solute, and the liquid that does the dissolving (in this case water) is the solvent. Water is an excellent solvent for many substances because of its polar bonds.

ACIDS

Substances that release hydrogen ions into solution are called acids.

$$\underset{\text{hydrochloric acid (strong acid)}}{HCl} \longrightarrow \underset{\text{hydrogen ion}}{H^+} + \underset{\text{chloride ion}}{Cl^-}$$

Many of the acids important in the cell are only partially dissociated, and they are therefore weak acids—for example, the carboxyl group (–COOH), which dissociates to give a hydrogen ion in solution

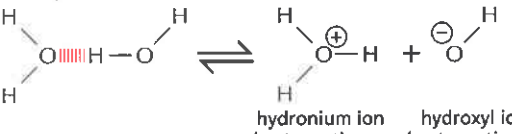


(weak acid)

Note that this is a reversible reaction.

HYDROGEN ION EXCHANGE

Positively charged hydrogen ions (H^+) can spontaneously move from one water molecule to another, thereby creating two ionic species.



often written as: $H_2O \rightleftharpoons \underset{\text{hydrogen ion}}{H^+} + \underset{\text{hydroxyl ion}}{OH^-}$

Since the process is rapidly reversible, hydrogen ions are continually shuttling between water molecules. Pure water contains a steady-state concentration of hydrogen ions and hydroxyl ions (both 10^{-7} M).

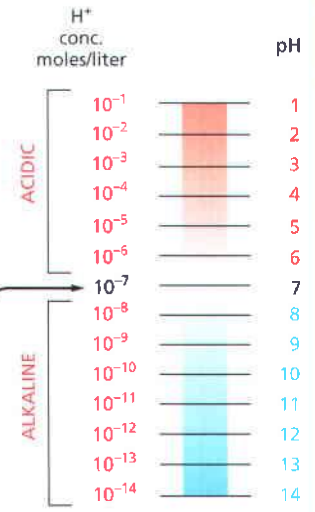
pH

The acidity of a solution is defined by the concentration of H^+ ions it possesses. For convenience we use the pH scale, where

$pH = -\log_{10}[H^+]$

For pure water

$[H^+] = 10^{-7}$ moles/liter



BASES

Substances that reduce the number of hydrogen ions in solution are called bases. Some bases, such as ammonia, combine directly with hydrogen ions.

$$\underset{\text{ammonia}}{NH_3} + \underset{\text{hydrogen ion}}{H^+} \longrightarrow \underset{\text{ammonium ion}}{NH_4^+}$$

Other bases, such as sodium hydroxide, reduce the number of H^+ ions indirectly, by making OH^- ions that then combine directly with H^+ ions to make H_2O.

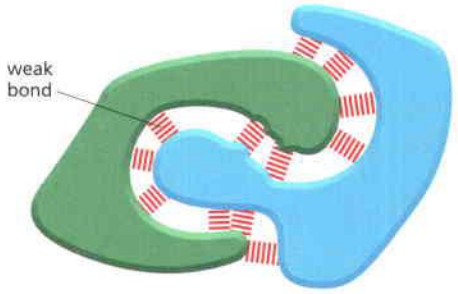
$$\underset{\text{sodium hydroxide (strong base)}}{NaOH} \longrightarrow \underset{\text{sodium ion}}{Na^+} + \underset{\text{hydroxyl ion}}{OH^-}$$

Many bases found in cells are partially dissociated and are termed weak bases. This is true of compounds that contain an amino group ($-NH_2$), which has a weak tendency to reversibly accept an H^+ ion from water, increasing the quantity of free OH^- ions.

$$-NH_2 + H^+ \rightleftharpoons -NH_3^+$$

WEAK CHEMICAL BONDS

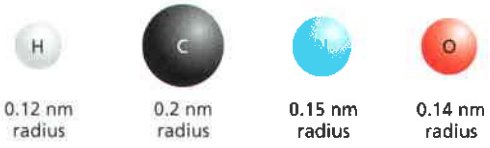
Organic molecules can interact with other molecules through three types of short-range attractive forces known as *noncovalent bonds:* van der Waals attractions, electrostatic attractions, and hydrogen bonds. The repulsion of hydrophobic groups from water is also important for ordering biological macromolecules.



Weak chemical bonds have less than 1/20 the strength of a strong covalent bond. They are strong enough to provide tight binding only when many of them are formed simultaneously.

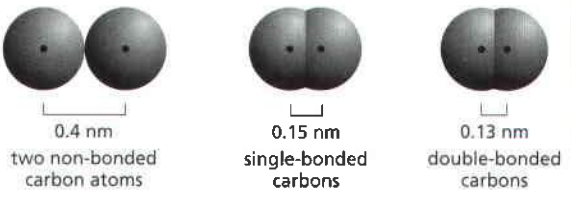
VAN DER WAALS ATTRACTIONS

If two atoms are too close together they repel each other very strongly. For this reason, an atom can often be treated as a sphere with a fixed radius. The characteristic "size" for each atom is specified by a unique van der Waals radius. The contact distance between any two noncovalently bonded atoms is the sum of their van der Waals radii.



At very short distances any two atoms show a weak bonding interaction due to their fluctuating electrical charges. The two atoms will be attracted to each other in this way until the distance between their nuclei is approximately equal to the sum of their van der Waals radii. Although they are individually very weak, van der Waals attractions can become important when two macromolecular surfaces fit very close together, because many atoms are involved.

Note that when two atoms form a covalent bond, the centers of the two atoms (the two atomic nuclei) are much closer together than the sum of the two van der Waals radii. Thus,



HYDROGEN BONDS

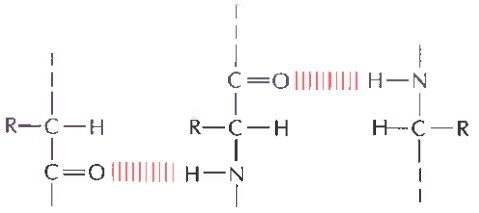
As already described for water (see Panel 2–2), hydrogen bonds form when a hydrogen atom is "sandwiched" between two electron-attracting atoms (usually oxygen or nitrogen).

Hydrogen bonds are strongest when the three atoms are in a straight line:

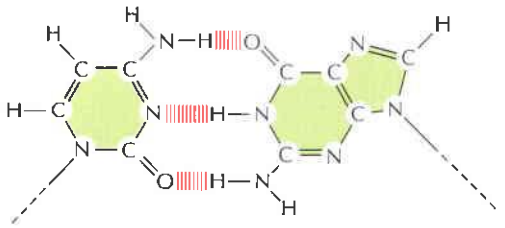


Examples in macromolecules:

Amino acids in polypeptide chains hydrogen-bonded together.

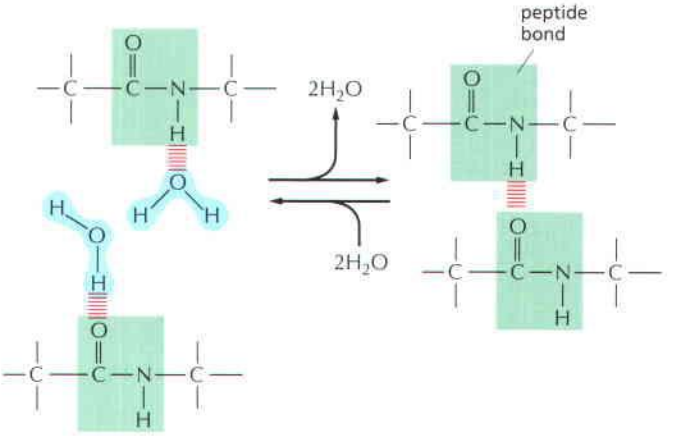


Two bases, G and C, hydrogen-bonded in DNA or RNA.

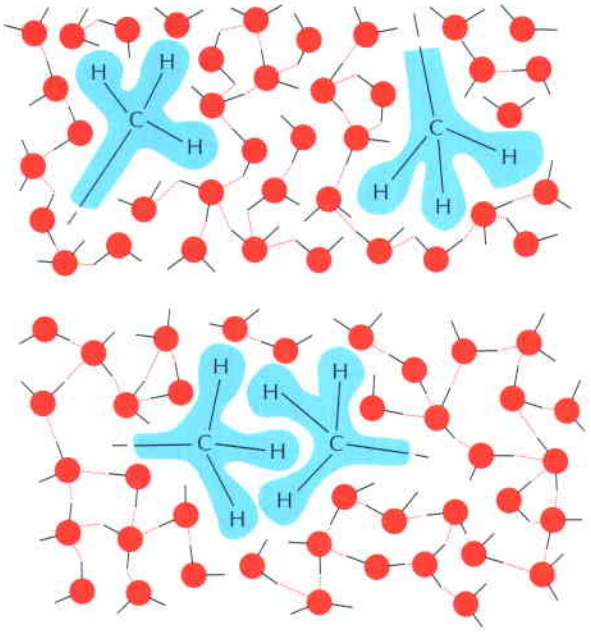


HYDROGEN BONDS IN WATER

Any molecules that can form hydrogen bonds to each other can alternatively form hydrogen bonds to water molecules. Because of this competition with water molecules, the hydrogen bonds formed between two molecules dissolved in water are relatively weak.



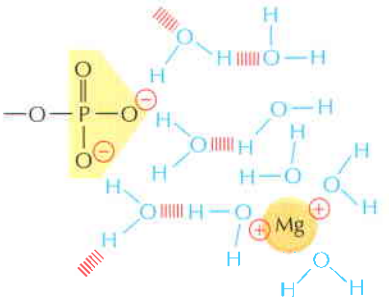
HYDROPHOBIC FORCES



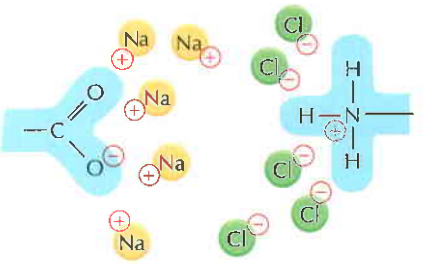
Water forces hydrophobic groups together, because doing so minimizes their disruptive effects on the hydrogen-bonded water network. Hydrophobic groups held together in this way are sometimes said to be held together by "hydrophobic bonds," even though the apparent attraction is actually caused by a repulsion from the water.

ELECTROSTATIC ATTRACTIONS IN AQUEOUS SOLUTIONS

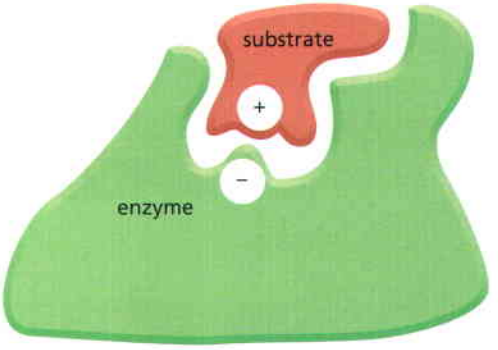
Charged groups are shielded by their interactions with water molecules. Electrostatic attractions are therefore quite weak in water.



Similarly, ions in solution can cluster around charged groups and further weaken these attractions.



Despite being weakened by water and salt, electrostatic attractions are very important in biological systems. For example, an enzyme that binds a positively charged substrate will often have a negatively charged amino acid side chain at the appropriate place.



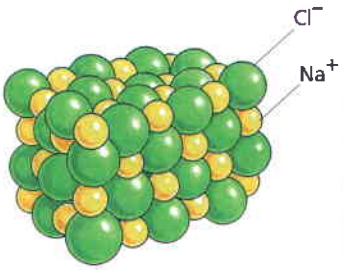
ELECTROSTATIC ATTRACTIONS

Attractive forces occur both between fully charged groups (ionic bond) and between the partially charged groups on polar molecules.

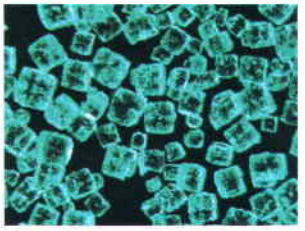


The force of attraction between the two charges, δ^+ and δ^-, falls off rapidly as the distance between the charges increases.

In the absence of water, electrostatic forces are very strong. They are responsible for the strength of such minerals as marble and agate, and for crystal formation in common table salt, NaCl.

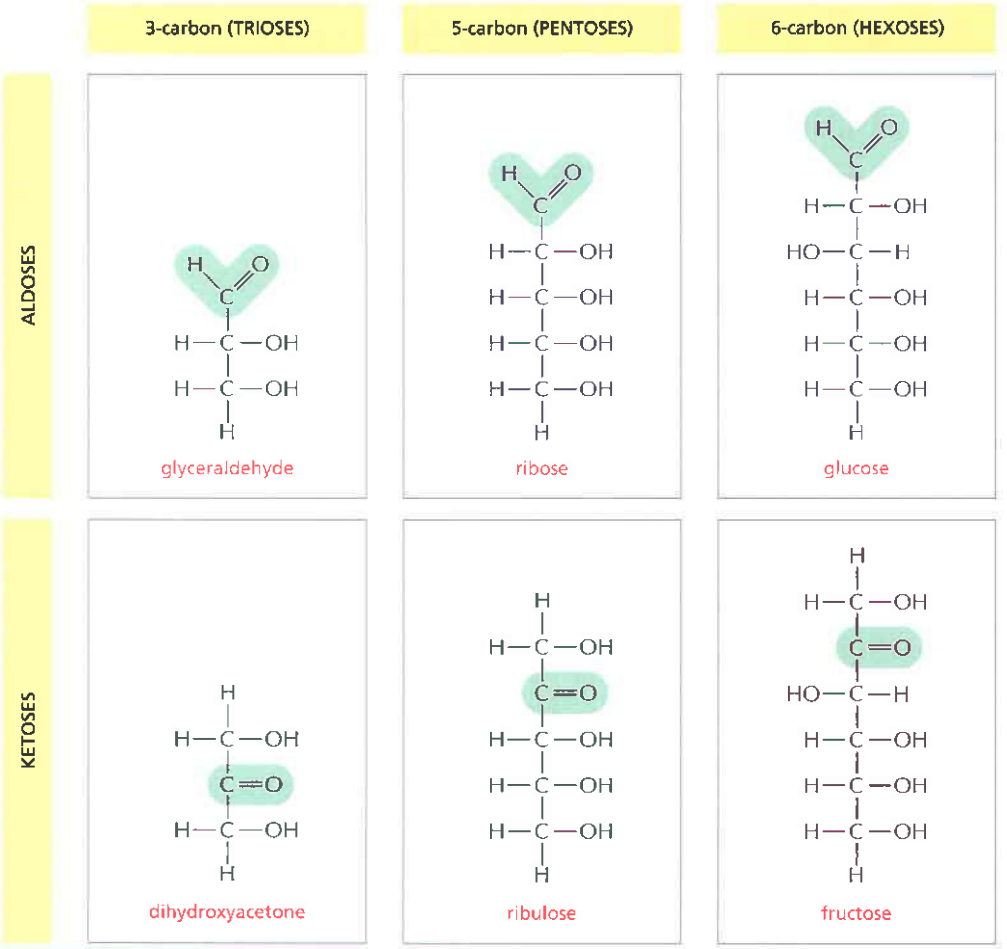


a crystal of salt, NaCl



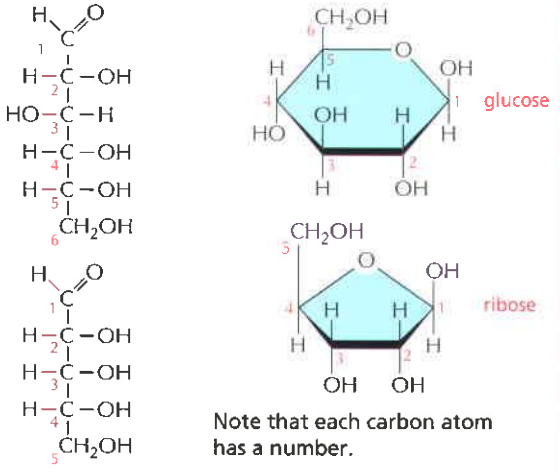
MONOSACCHARIDES

Monosaccharides usually have the general formula $(CH_2O)_n$, where n can be 3, 4, 5, 6, 7, or 8, and have two or more hydroxyl groups. They either contain an aldehyde group ($-C\overset{O}{\underset{H}{<}}$) and are called aldoses or a ketone group ($>C=O$) and are called ketoses.



RING FORMATION

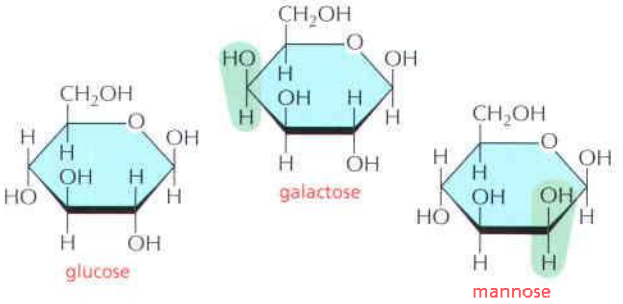
In aqueous solution, the aldehyde or ketone group of a sugar molecule tends to react with a hydroxyl group of the same molecule, thereby closing the molecule into a ring.



Note that each carbon atom has a number.

ISOMERS

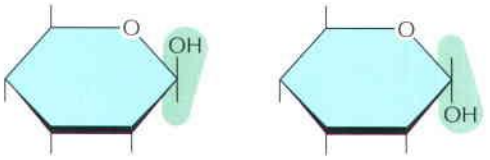
Many monosaccharides differ only in the spatial arrangement of atoms—that is, they are isomers. For example, glucose, galactose, and mannose have the same formula ($C_6H_{12}O_6$) but differ in the arrangement of groups around one or two carbon atoms.



These small differences make only minor changes in the chemical properties of the sugars. But they are recognized by enzymes and other proteins and therefore can have important biological effects.

α AND β LINKS

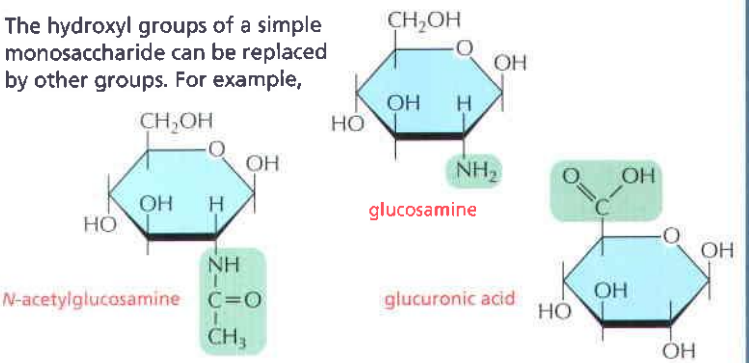
The hydroxyl group on the carbon that carries the aldehyde or ketone can rapidly change from one position to the other. These two positions are called α and β.



As soon as one sugar is linked to another, the α or β form is frozen.

SUGAR DERIVATIVES

The hydroxyl groups of a simple monosaccharide can be replaced by other groups. For example,



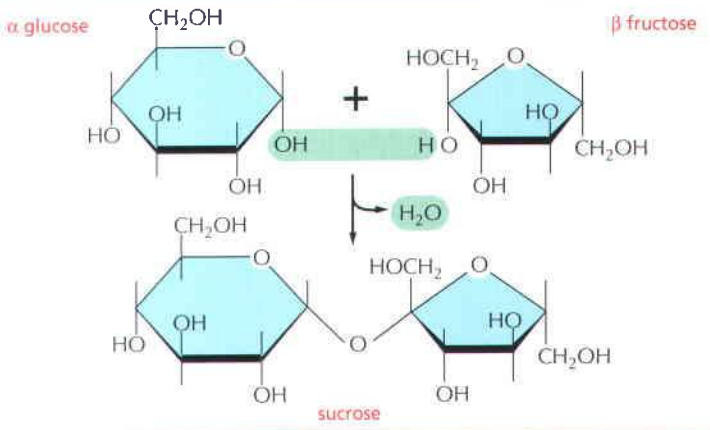
DISACCHARIDES

The carbon that carries the aldehyde or the ketone can react with any hydroxyl group on a second sugar molecule to form a disaccharide. The linkage is called a glycosidic bond.

Three common disaccharides are

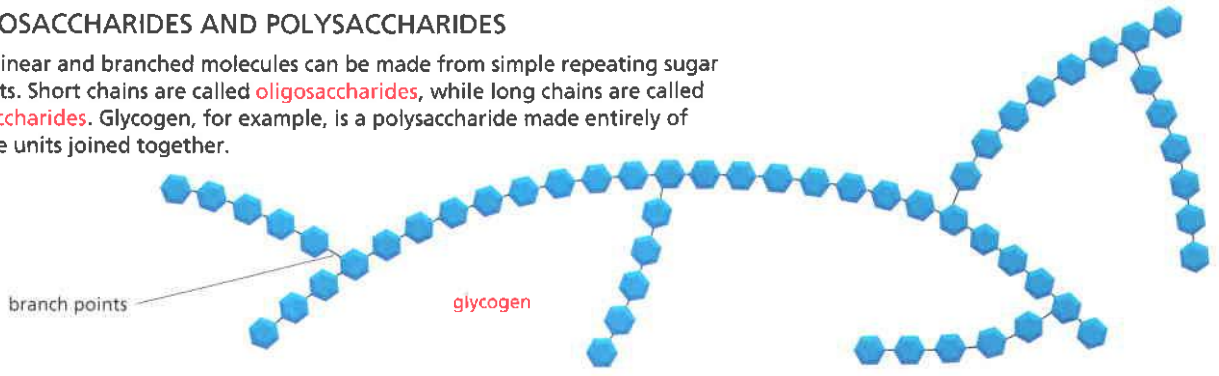
- maltose (glucose + glucose)
- lactose (galactose + glucose)
- sucrose (glucose + fructose)

The reaction forming sucrose is shown here.



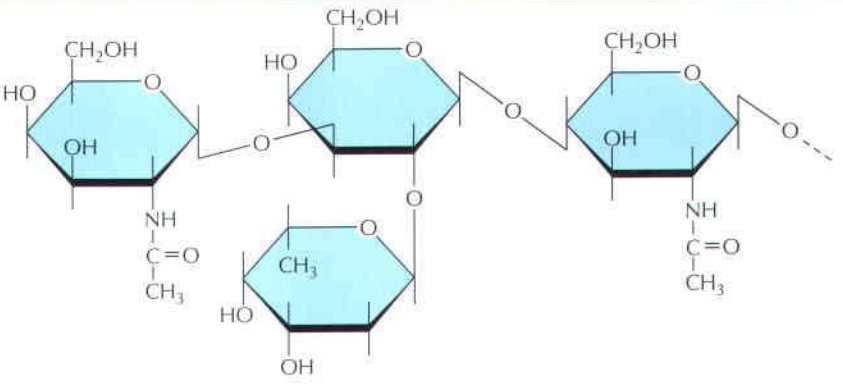
OLIGOSACCHARIDES AND POLYSACCHARIDES

Large linear and branched molecules can be made from simple repeating sugar subunits. Short chains are called oligosaccharides, while long chains are called polysaccharides. Glycogen, for example, is a polysaccharide made entirely of glucose units joined together.



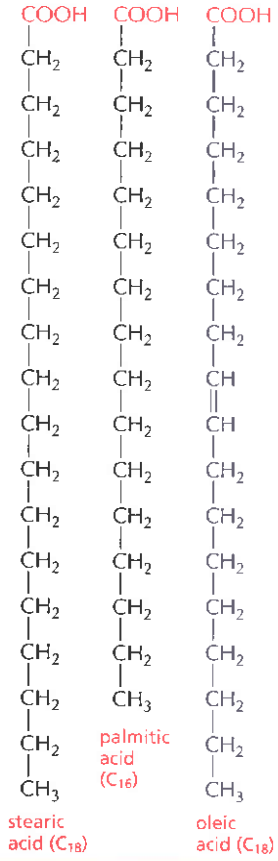
COMPLEX OLIGOSACCHARIDES

In many cases a sugar sequence is nonrepetitive. Many different molecules are possible. Such complex oligosaccharides are usually linked to proteins or to lipids, as is this oligosaccharide, which is part of a cell-surface molecule that defines a particular blood group.



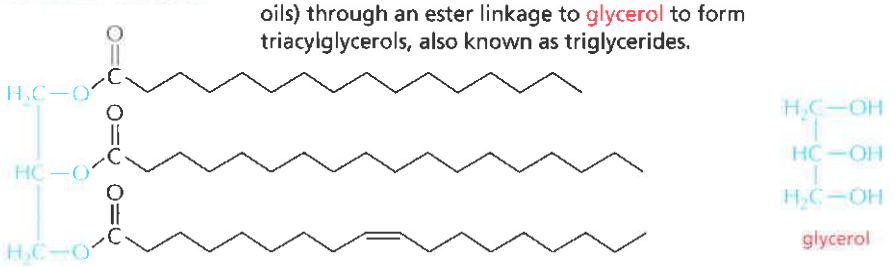
COMMON FATTY ACIDS

These are carboxylic acids with long hydrocarbon tails.

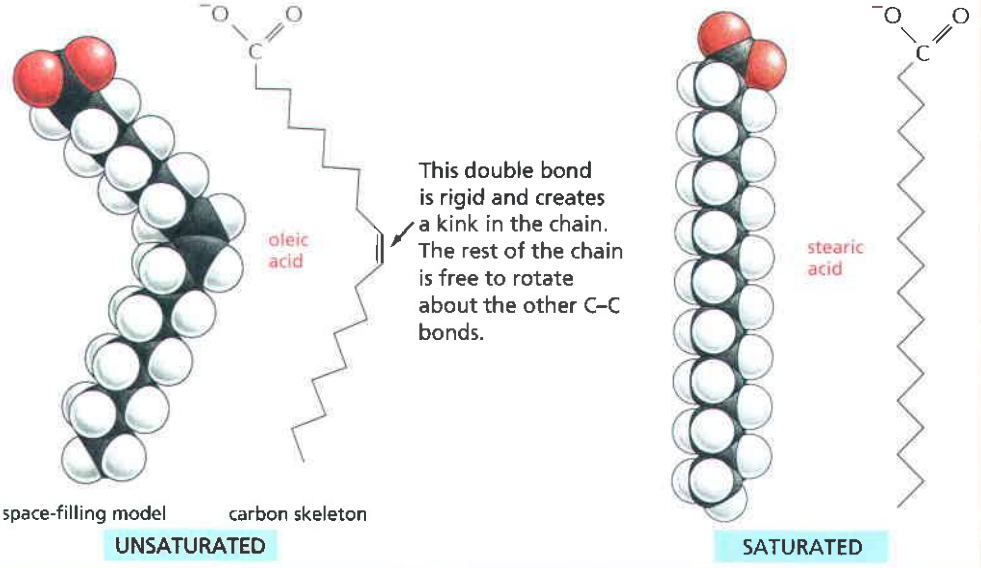


TRIACYLGLYCEROLS

Fatty acids are stored as an energy reserve (fats and oils) through an ester linkage to glycerol to form triacylglycerols, also known as triglycerides.

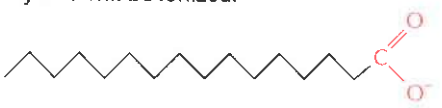


Hundreds of different kinds of fatty acids exist. Some have one or more double bonds in their hydrocarbon tail and are said to be unsaturated. Fatty acids with no double bonds are saturated.

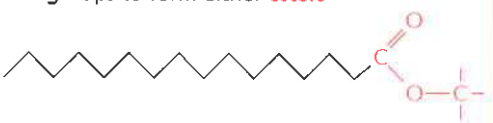


CARBOXYL GROUP

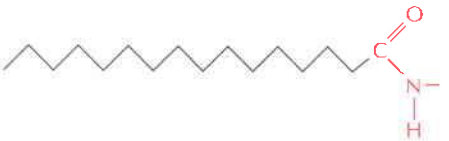
If free, the carboxyl group of a fatty acid will be ionized.



But more usually it is linked to other groups to form either esters

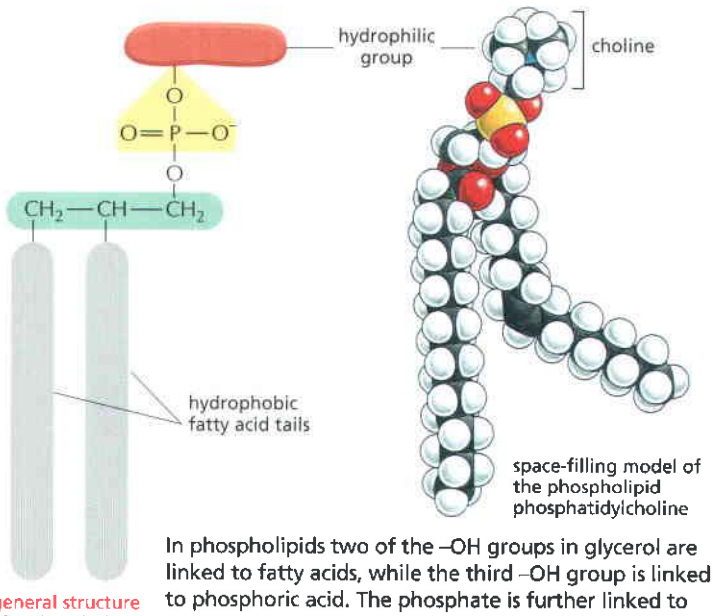


or amides.



PHOSPHOLIPIDS

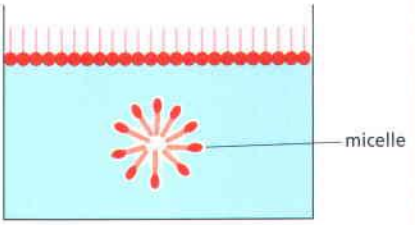
Phospholipids are the major constituents of cell membranes.



In phospholipids two of the –OH groups in glycerol are linked to fatty acids, while the third –OH group is linked to phosphoric acid. The phosphate is further linked to one of a variety of small polar groups (alcohols).

LIPID AGGREGATES

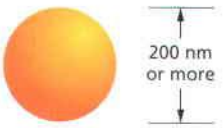
Fatty acids have a hydrophilic head and a hydrophobic tail.



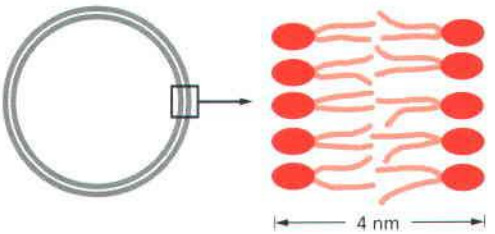
In water they can form a surface film or form small micelles.

Their derivatives can form larger aggregates held together by hydrophobic forces:

Triglycerides can form large spherical fat droplets in the cell cytoplasm.

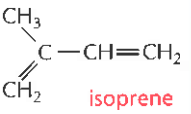


Phospholipids and glycolipids form self-sealing lipid bilayers that are the basis for all cell membranes.



OTHER LIPIDS

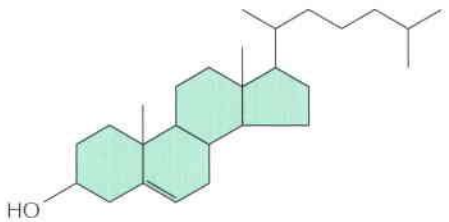
Lipids are defined as the water-insoluble molecules in cells that are soluble in organic solvents. Two other common types of lipids are steroids and polyisoprenoids. Both are made from isoprene units.



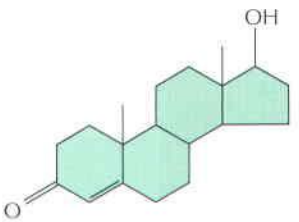
isoprene

STEROIDS

Steroids have a common multiple-ring structure.



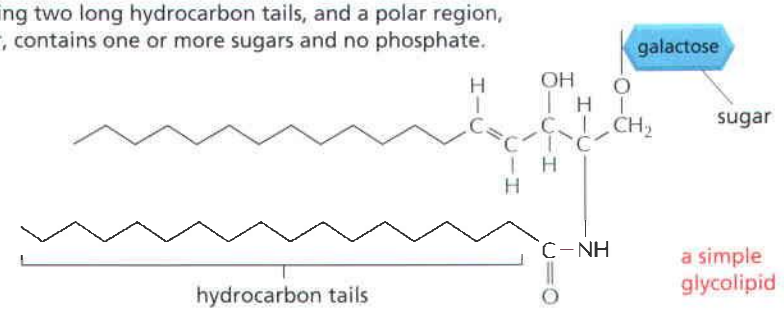
cholesterol—found in many membranes



testosterone—male steroid hormone

GLYCOLIPIDS

Like phospholipids, these compounds are composed of a hydrophobic region, containing two long hydrocarbon tails, and a polar region, which, however, contains one or more sugars and no phosphate.



a simple glycolipid

POLYISOPRENOIDS

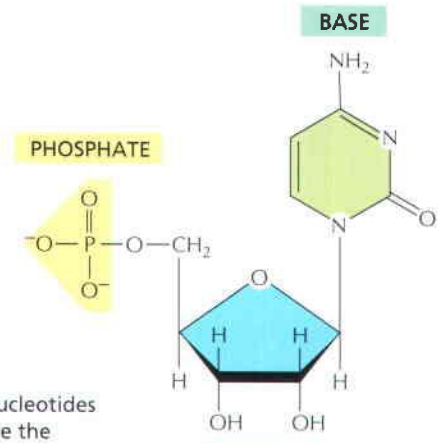
long-chain polymers of isoprene



dolichol phosphate—used to carry activated sugars in the membrane-associated synthesis of glycoproteins and some polysaccharides

NUCLEOTIDES

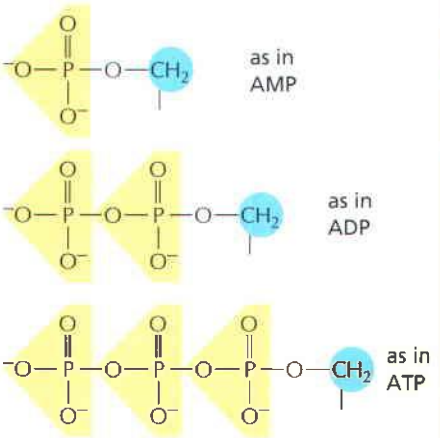
A nucleotide consists of a nitrogen-containing base, a five-carbon sugar, and one or more phosphate groups.



Nucleotides are the subunits of the nucleic acids.

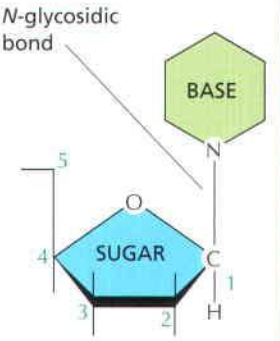
PHOSPHATES

The phosphates are normally joined to the C5 hydroxyl of the ribose or deoxyribose sugar (designated 5'). Mono-, di-, and triphosphates are common.



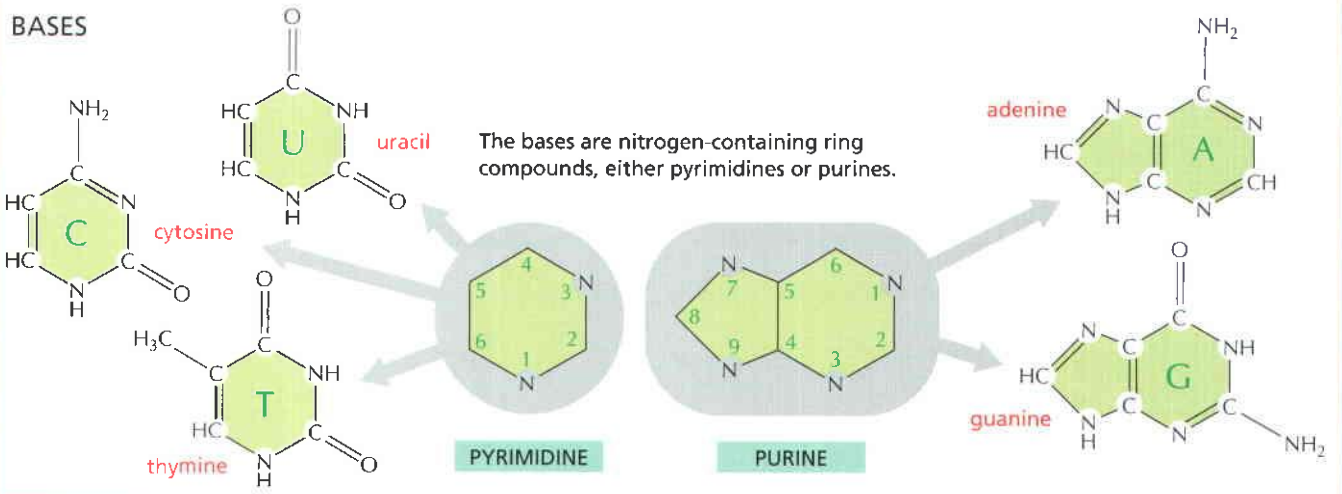
The phosphate makes a nucleotide negatively charged.

BASIC SUGAR LINKAGE



The base is linked to the same carbon (C1) used in sugar–sugar bonds.

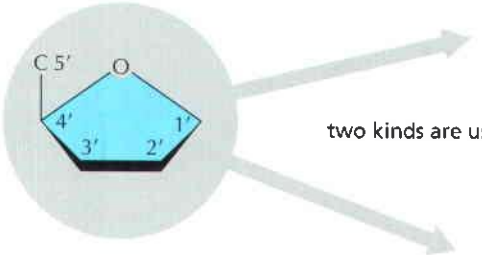
BASES



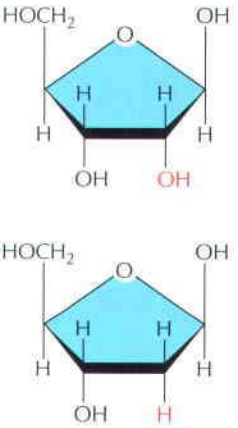
The bases are nitrogen-containing ring compounds, either pyrimidines or purines.

SUGARS

PENTOSE
a five-carbon sugar



two kinds are used



β-D-ribose
used in ribonucleic acid

β-D-2-deoxyribose
used in deoxyribonucleic acid

Each numbered carbon on the sugar of a nucleotide is followed by a prime mark; therefore, one speaks of the "5-prime carbon," etc.

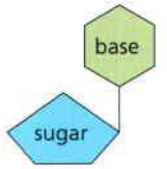
NOMENCLATURE

A nucleoside or nucleotide is named according to its nitrogenous base.

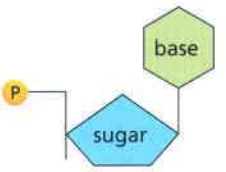
BASE	NUCLEOSIDE	ABBR.
adenine	adenosine	A
guanine	guanosine	G
cytosine	cytidine	C
uracil	uridine	U
thymine	thymidine	T

Single letter abbreviations are used variously as shorthand for (1) the base alone, (2) the nucleoside, or (3) the whole nucleotide—the context will usually make clear which of the three entities is meant. When the context is not sufficient, we will add the terms "base", "nucleoside", "nucleotide", or—as in the examples below—use the full 3-letter nucleotide code.

AMP = adenosine monophosphate
dAMP = deoxyadenosine monophosphate
UDP = uridine diphosphate
ATP = adenosine triphosphate



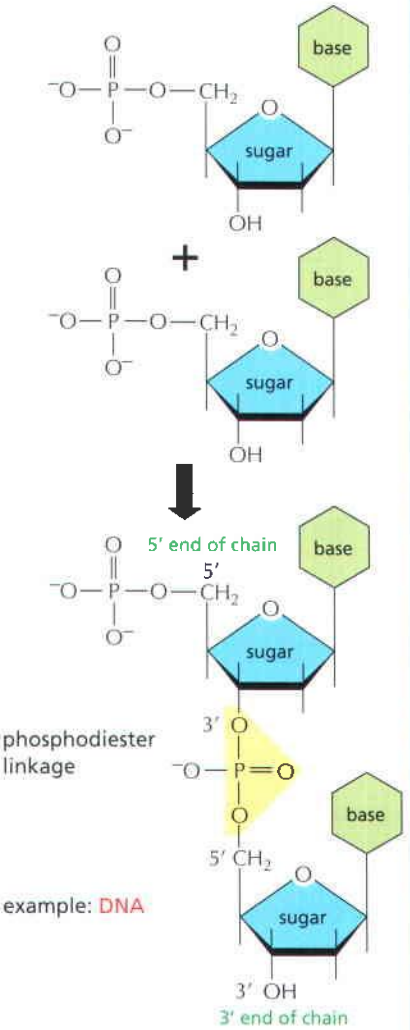
BASE + SUGAR = NUCLEOSIDE



BASE + SUGAR + PHOSPHATE = NUCLEOTIDE

NUCLEIC ACIDS

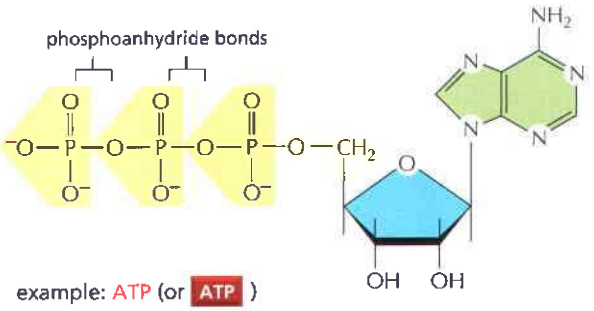
Nucleotides are joined together by a phosphodiester linkage between 5′ and 3′ carbon atoms to form nucleic acids. The linear sequence of nucleotides in a nucleic acid chain is commonly abbreviated by a one-letter code, A—G—C—T—T—A—C—A, with the 5′ end of the chain at the left.



example: DNA

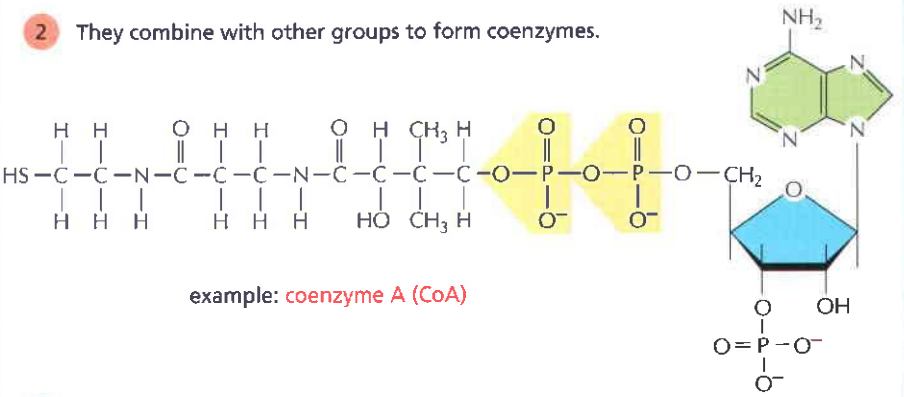
NUCLEOTIDES HAVE MANY OTHER FUNCTIONS

1 They carry chemical energy in their easily hydrolyzed phosphoanhydride bonds.



example: ATP (or ATP)

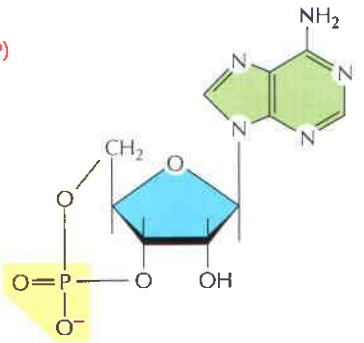
2 They combine with other groups to form coenzymes.



example: coenzyme A (CoA)

3 They are used as specific signaling molecules in the cell.

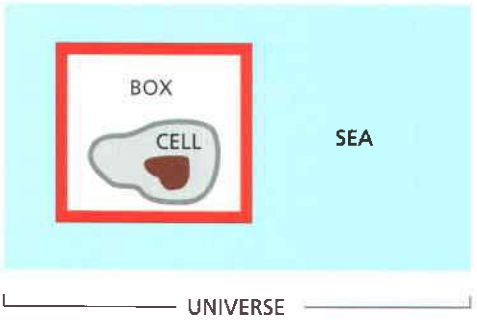
example: cyclic AMP (cAMP)



THE IMPORTANCE OF FREE ENERGY FOR CELLS

Life is possible because of the complex network of interacting chemical reactions occurring in every cell. In viewing the metabolic pathways that comprise this network, one might suspect that the cell has had the ability to evolve an enzyme to carry out any reaction that it needs. But this is not so. Although enzymes are powerful catalysts, they can speed up only those reactions that are thermodynamically possible; other reactions proceed in cells only because they are *coupled* to very favorable reactions that drive them. The question of whether a reaction can occur spontaneously, or instead needs to be coupled to another reaction, is central to cell biology. The answer is obtained by reference to a quantity called the *free energy:* the total change in free energy during a set of reactions determines whether or not the entire reaction sequence can occur. In this panel we shall explain some of the fundamental ideas—derived from a special branch of chemistry and physics called *thermodynamics*—that are required for understanding what free energy is and why it is so important to cells.

ENERGY RELEASED BY CHANGES IN CHEMICAL BONDING IS CONVERTED INTO HEAT



An *enclosed system* is defined as a collection of molecules that does not exchange matter with the rest of the universe (for example, the "cell in a box" shown above). Any such system will contain molecules with a total energy E. This energy will be distributed in a variety of ways: some as the translational energy of the molecules, some as their vibrational and rotational energies, but most as the bonding energies between the individual atoms that make up the molecules. Suppose that a reaction occurs in the system. The first law of thermodynamics places a constraint on what types of reactions are possible: it states that "in any process, the total energy of the universe remains constant." For example, suppose that reaction A → B occurs somewhere in the box and releases a great deal of chemical bond energy. This energy will initially increase the intensity of molecular motions (translational, vibrational, and rotational) in the system, which is equivalent to raising its temperature. However, these increased motions will soon be transferred out of the system by a series of molecular collisions that heat up first the walls of the box and then the outside world (represented by the sea in our example). In the end, the system returns to its initial temperature, by which time all the chemical bond energy released in the box has been converted into heat energy and transferred out of the box to the surroundings. According to the first law, the change in the energy in the box (ΔE_{box}, which we shall denote as ΔE) must be equal and opposite to the amount of heat energy transferred, which we shall designate as h: that is, $\Delta E = -h$. Thus, the energy in the box (E) decreases when heat leaves the system.

E also can change during a reaction as a result of work being done on the outside world. For example, suppose that there is a small increase in the volume (ΔV) of the box during a reaction. Since the walls of the box must push against the constant pressure (P) in the surroundings in order to expand, this does work on the outside world and requires energy. The energy used is $P(\Delta V)$, which according to the first law must decrease the energy in the box (E) by the same amount. In most reactions chemical bond energy is converted into both work and heat. *Enthalpy* (H) is a composite function that includes both of these ($H = E + PV$). To be rigorous, it is the change in enthalpy (ΔH) in an enclosed system, and not the change in energy, that is equal to the heat transferred to the outside world during a reaction. Reactions in which H decreases release heat to the surroundings and are said to be "exothermic," while reactions in which H increases absorb heat from the surroundings and are said to be "endothermic." Thus, $-h = \Delta H$. However, the volume change is negligible in most biological reactions, so to a good approximation

$$-h = \Delta H \cong \Delta E$$

THE SECOND LAW OF THERMODYNAMICS

Consider a container in which 1000 coins are all lying heads up. If the container is shaken vigorously, subjecting the coins to the types of random motions that all molecules experience due to their frequent collisions with other molecules, one will end up with about half the coins oriented heads down. The reason for this reorientation is that there is only a single way in which the original orderly state of the coins can be reinstated (every coin must lie heads up), whereas there are many different ways (about 10^{298}) to achieve a disorderly state in which there is an equal mixture of heads and tails; in fact, there are more ways to achieve a 50-50 state than to achieve any other state. Each state has a probability of occurrence that is proportional to the number of ways it can be realized. The second law of thermodynamics states that "systems will change spontaneously from states of lower probability to states of higher probability." Since states of lower probability are more "ordered" than states of high probability, the second law can be restated: "the universe constantly changes so as to become more disordered."

THE ENTROPY, *S*

The second law (but not the first law) allows one to predict the *direction* of a particular reaction. But to make it useful for this purpose, one needs a convenient measure of the probability or, equivalently, the degree of disorder of a state. The entropy (*S*) is such a measure. It is a logarithmic function of the probability such that the *change in entropy* (ΔS) that occurs when the reaction A → B converts one mole of A into one mole of B is

$$\Delta S = R \ln p_B / p_A$$

where p_A and p_B are the probabilities of the two states A and B, R is the gas constant (2 cal deg^{-1} mole^{-1}), and ΔS is measured in entropy units (eu). In our initial example of 1000 coins, the relative probability of all heads (state A) versus half heads and half tails (state B) is equal to the ratio of the number of different ways that the two results can be obtained. One can calculate that $p_A = 1$ and $p_B = 1000!/(500! \times 500!) = 10^{299}$. Therefore, the entropy change for the reorientation of the coins when their container is vigorously shaken and an equal mixture of heads and tails is obtained is $R \ln (10^{298})$, or about 1370 eu per mole of such containers (6×10^{23} containers). We see that, because ΔS defined above is positive for the transition from state A to state B ($p_B/p_A > 1$), reactions with a large *increase* in S (that is, for which $\Delta S > 0$) are favored and will occur spontaneously.

As discussed in Chapter 2, heat energy causes the random commotion of molecules. Because the transfer of heat from an enclosed system to its surroundings increases the number of different arrangements that the molecules in the outside world can have, it increases their entropy. It can be shown that the release of a fixed quantity of heat energy has a greater disordering effect at low temperature than at high temperature, and that the value of ΔS for the surroundings, as defined above (ΔS_{sea}), is precisely equal to h, the amount of heat transferred to the surroundings from the system, divided by the absolute temperature (T):

$$\Delta S_{sea} = h/T$$

THE GIBBS FREE ENERGY, *G*

When dealing with an enclosed biological system, one would like to have a simple way of predicting whether a given reaction will or will not occur spontaneously in the system. We have seen that the crucial question is whether the entropy change for the universe is positive or negative when that reaction occurs. In our idealized system, the cell in a box, there are two separate components to the entropy change of the universe—the entropy change for the system enclosed in the box and the entropy change for the surrounding "sea"—and both must be added together before any prediction can be made. For example, it is possible for a reaction to absorb heat and thereby decrease the entropy of the sea ($\Delta S_{sea} < 0$) and at the same time to cause such a large degree of disordering inside the box ($\Delta S_{box} > 0$) that the total $\Delta S_{universe} = \Delta S_{sea} + \Delta S_{box}$ is greater than 0. In this case the reaction will occur spontaneously, even though the sea gives up heat to the box during the reaction. An example of such a reaction is the dissolving of sodium chloride in a beaker containing water (the "box"), which is a spontaneous process even though the temperature of the water drops as the salt goes into solution.

Chemists have found it useful to define a number of new "composite functions" that describe *combinations* of physical properties of a system. The properties that can be combined include the temperature (T), pressure (P), volume (V), energy (E), and entropy (S). The enthalpy (H) is one such composite function. But by far the most useful composite function for biologists is the *Gibbs free energy, G*. It serves as an accounting device that allows one to deduce the entropy change of the universe resulting from a chemical reaction in the box, while avoiding any separate consideration of the entropy change in the sea. The definition of G is

$$G = H - TS$$

where, for a box of volume V, H is the enthalpy described above ($E + PV$), T is the absolute temperature, and S is the entropy. Each of these quantities applies to the inside of the box only. The change in free energy during a reaction in the box (the G of the products minus the G of the starting materials) is denoted as ΔG and, as we shall now demonstrate, it is a direct measure of the amount of disorder that is created in the universe when the reaction occurs.

At constant temperature the change in free energy (ΔG) during a reaction equals $\Delta H - T\Delta S$. Remembering that $\Delta H = -h$, the heat absorbed from the sea, we have

$$-\Delta G = -\Delta H + T\Delta S$$
$$-\Delta G = h + T\Delta S, \text{ so } -\Delta G/T = h/T + \Delta S$$

But h/T is equal to the entropy change of the sea (ΔS_{sea}), and the ΔS in the above equation is ΔS_{box}. Therefore

$$-\Delta G/T = \Delta S_{sea} + \Delta S_{box} = \Delta S_{universe}$$

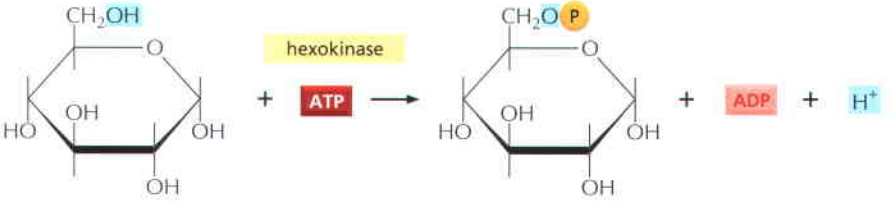
We conclude that the free-energy change is a direct measure of the entropy change of the universe. A reaction will proceed in the direction that causes the change in the free energy (ΔG) to be less than zero, because in this case there will be a positive entropy change in the universe when the reaction occurs.

For a complex set of coupled reactions involving many different molecules, the total free-energy change can be computed simply by adding up the free energies of all the different molecular species after the reaction and comparing this value with the sum of free energies before the reaction; for common substances the required free-energy values can be found from published tables. In this way one can predict the direction of a reaction and thereby readily check the feasibility of any proposed mechanism. Thus, for example, from the observed values for the magnitude of the electrochemical proton gradient across the inner mitochondrial membrane and the ΔG for ATP hydrolysis inside the mitochondrion, one can be certain that ATP synthase requires the passage of more than one proton for each molecule of ATP that it synthesizes.

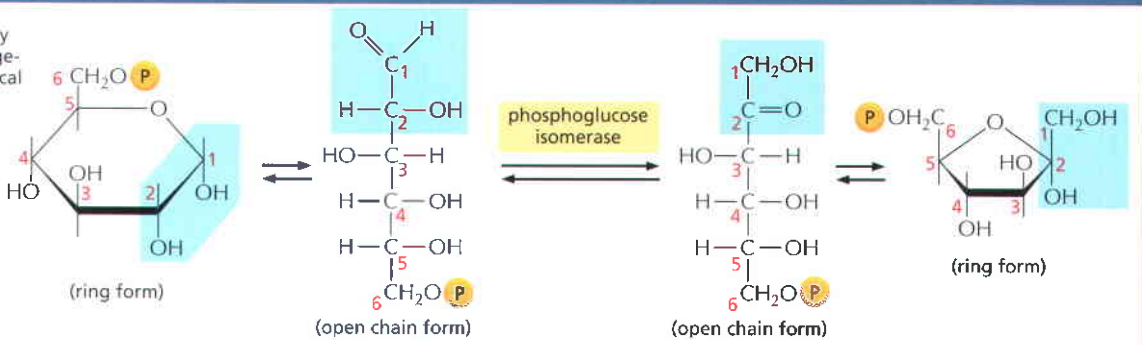
The value of ΔG for a reaction is a direct measure of how far the reaction is from equilibrium. The large negative value for ATP hydrolysis in a cell merely reflects the fact that cells keep the ATP hydrolysis reaction as much as 10 orders of magnitude away from equilibrium. If a reaction reaches equilibrium, $\Delta G = 0$, the reaction then proceeds at precisely equal rates in the forward and backward direction. For ATP hydrolysis, equilibrium is reached when the vast majority of the ATP has been hydrolyzed, as occurs in a dead cell.

For each step, the part of the molecule that undergoes a change is shadowed in blue, and the name of the enzyme that catalyzes the reaction is in a yellow box.

STEP 1 Glucose is phosphorylated by ATP to form a sugar phosphate. The negative charge of the phosphate prevents passage of the sugar phosphate through the plasma membrane, trapping glucose inside the cell.



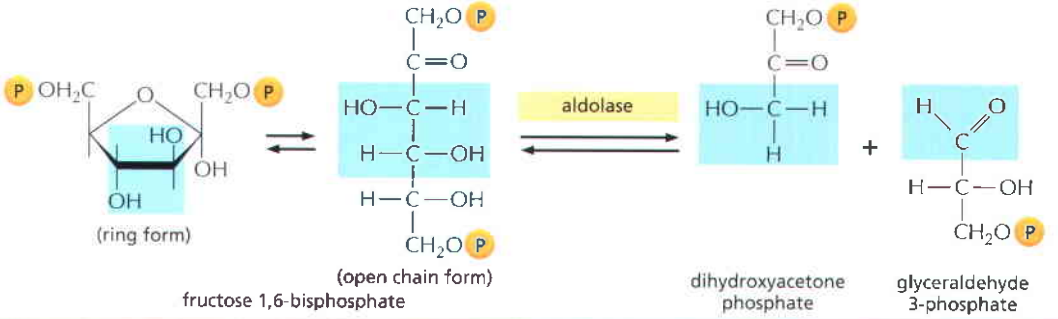
STEP 2 A readily reversible rearrangement of the chemical structure (isomerization) moves the carbonyl oxygen from carbon 1 to carbon 2, forming a ketose from an aldose sugar. (See Panel 2–4.)



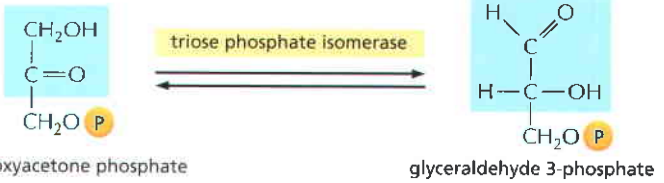
STEP 3 The new hydroxyl group on carbon 1 is phosphorylated by ATP, in preparation for the formation of two three-carbon sugar phosphates. The entry of sugars into glycolysis is controlled at this step, through regulation of the enzyme *phosphofructokinase*.



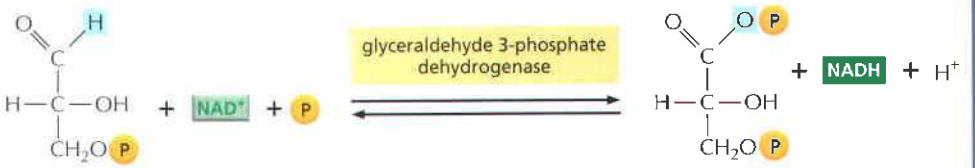
STEP 4 The six-carbon sugar is cleaved to produce two three-carbon molecules. Only the glyceraldehyde 3-phosphate can proceed immediately through glycolysis.



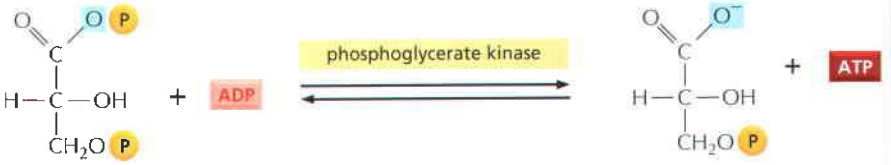
STEP 5 The other product of step 4, dihydroxyacetone phosphate, is isomerized to form glyceraldehyde 3-phosphate.



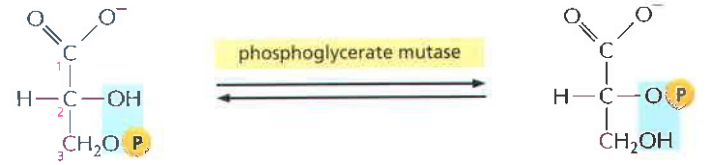
STEP 6 The two molecules of glyceraldehyde 3-phosphate are oxidized. The energy generation phase of glycolysis begins, as NADH and a new high-energy anhydride linkage to phosphate are formed (see Figure 2–73).



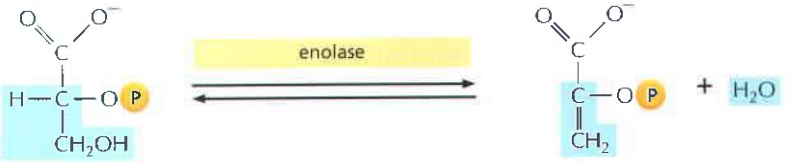
STEP 7 The transfer to ADP of the high-energy phosphate group that was generated in step 6 forms ATP.



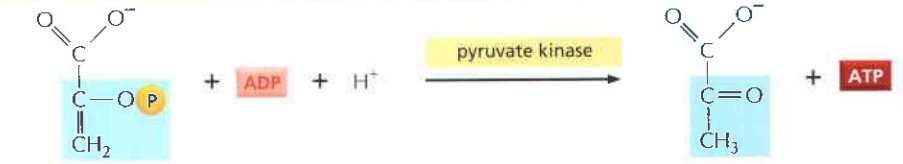
STEP 8 The remaining phosphate ester linkage in 3-phosphoglycerate, which has a relatively low free energy of hydrolysis, is moved from carbon 3 to carbon 2 to form 2-phosphoglycerate.



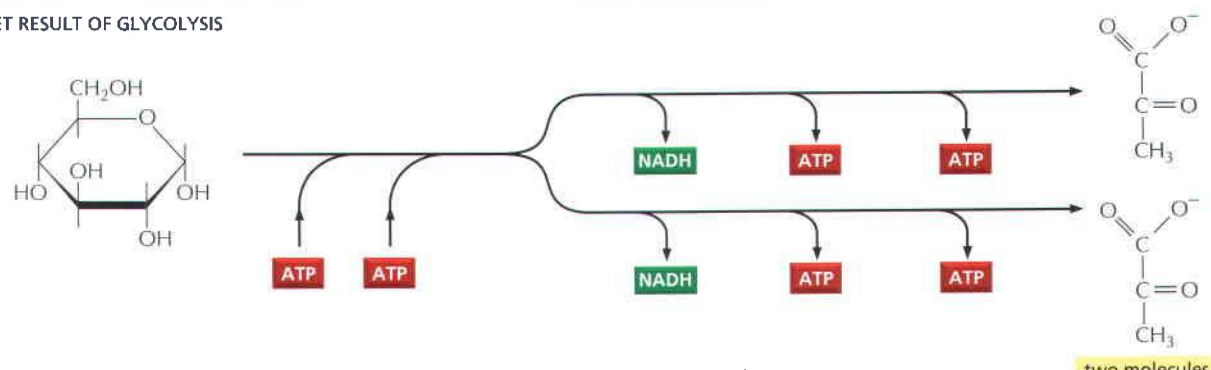
STEP 9 The removal of water from 2-phosphoglycerate creates a high-energy enol phosphate linkage.

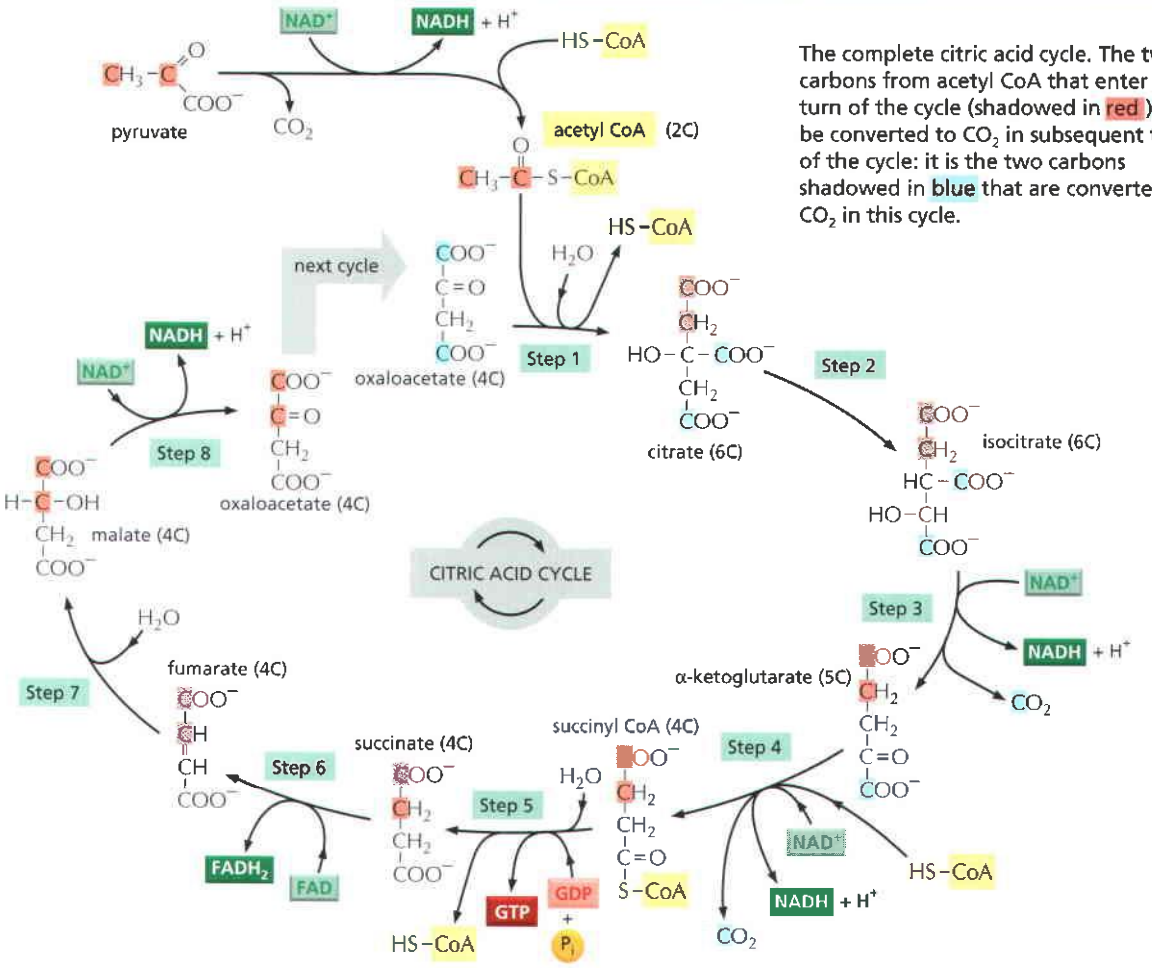


STEP 10 The transfer to ADP of the high-energy phosphate group that was generated in step 9 forms ATP, completing glycolysis.



NET RESULT OF GLYCOLYSIS

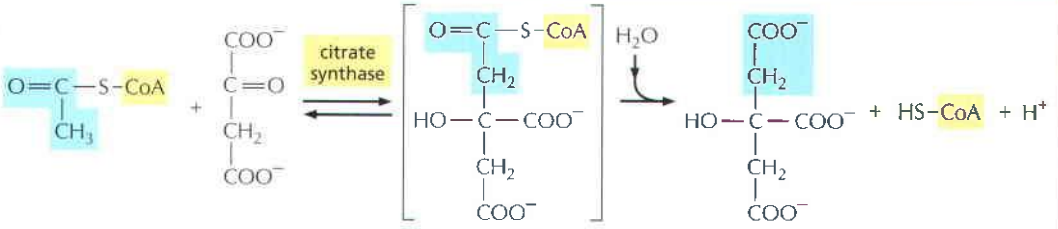




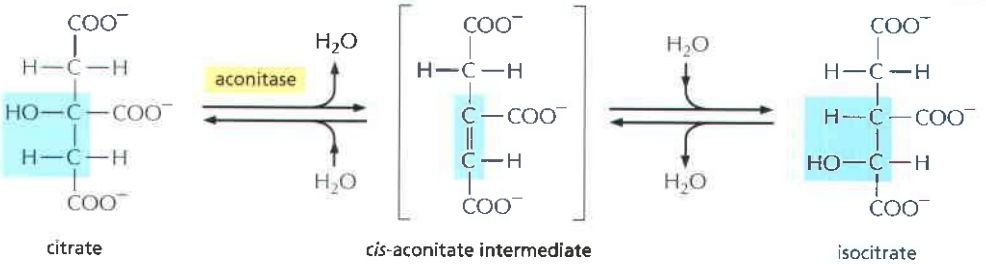
The complete citric acid cycle. The two carbons from acetyl CoA that enter this turn of the cycle (shadowed in red) will be converted to CO_2 in subsequent turns of the cycle: it is the two carbons shadowed in blue that are converted to CO_2 in this cycle.

Details of the eight steps are shown below. For each step, the part of the molecule that undergoes a change is shadowed in blue, and the name of the enzyme that catalyzes the reaction is in a yellow box.

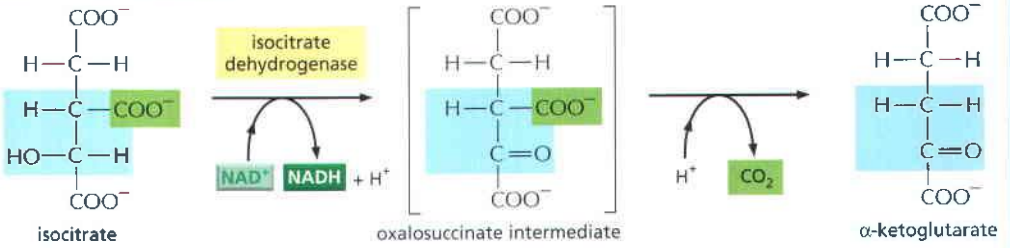
STEP 1 After the enzyme removes a proton from the CH_3 group on acetyl CoA, the negatively charged CH_2^- forms a bond to a carbonyl carbon of oxaloacetate. The subsequent loss by hydrolysis of the coenzyme A (CoA) drives the reaction strongly forward.



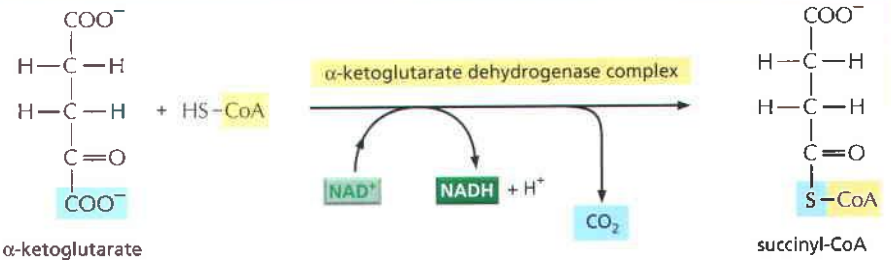
STEP 2 An isomerization reaction, in which water is first removed and then added back, moves the hydroxyl group from one carbon atom to its neighbor.



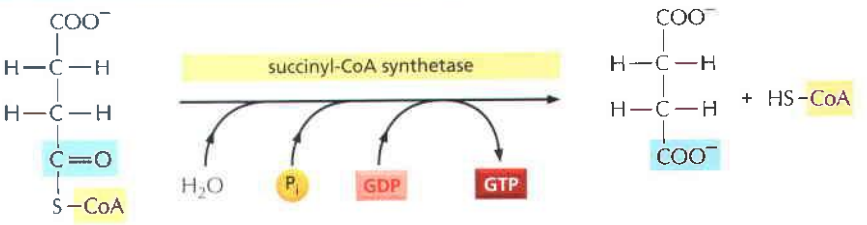
STEP 3 In the first of four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to a carbonyl group. The immediate product is unstable, losing CO_2 while still bound to the enzyme.



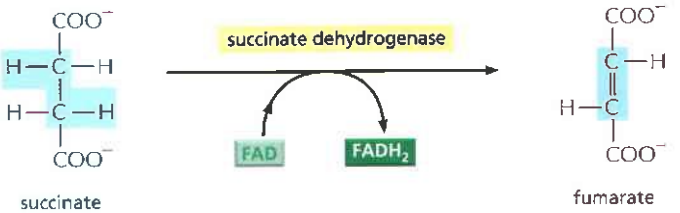
STEP 4 The *α-ketoglutarate dehydrogenase complex* closely resembles the large enzyme complex that converts pyruvate to acetyl CoA (*pyruvate dehydrogenase*). It likewise catalyzes an oxidation that produces NADH, CO_2, and a high-energy thioester bond to coenzyme A (CoA).



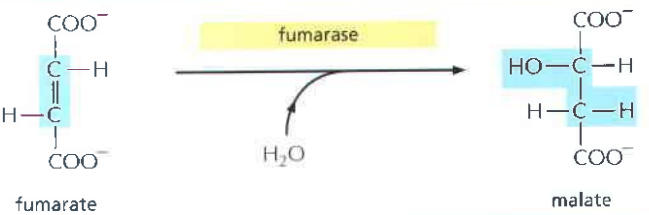
STEP 5 A phosphate molecule from solution displaces the CoA, forming a high-energy phosphate linkage to succinate. This phosphate is then passed to GDP to form GTP. (In bacteria and plants, ATP is formed instead.)



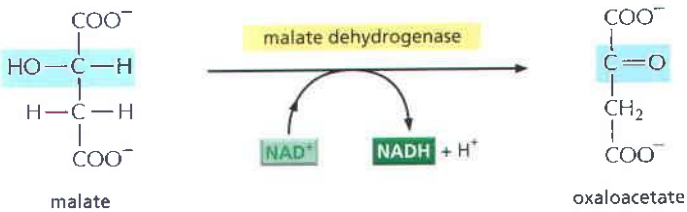
STEP 6 In the third oxidation step in the cycle, FAD removes two hydrogen atoms from succinate.



STEP 7 The addition of water to fumarate places a hydroxyl group next to a carbonyl carbon.



STEP 8 In the last of four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to a carbonyl group, regenerating the oxaloacetate needed for step 1.



REFERENCES

General

Berg, JM, Tymoczko, JL & Stryer L (2006) Biochemistry, 6th ed. New York: WH Freeman.
Garrett RH & Grisham CM (2005) Biochemistry, 3rd ed. Philadelphia: Thomson Brooks/Cole.
Horton HR, Moran LA, Scrimgeour et al (2005) Principles of Biochemistry 4th ed. Upper Saddle River, NJ: Prentice Hall.
Nelson DL & Cox MM (2004) Lehninger Principles of Biochemistry, 4th ed. New York: Worth.
Nicholls DG & Ferguson SJ (2002) Bioenergetics, 3rd ed. New York: Academic Press
Mathews CK, van Holde KE & Ahern K-G (2000) Biochemistry, 3rd ed. San Francisco: Benjamin Cummings.
Moore JA (1993) Science As a Way of Knowing. Cambridge, MA: Harvard University Press.
Voet D, Voet JG & Pratt CM (2004) Fundamentals of Biochemistry, 2nd ed. New York: Wiley.

The Chemical Components of a Cell

Abeles RH, Frey PA & Jencks WP (1992) Biochemistry. Boston: Jones & Bartlett.
Atkins PW (1996) Molecules. New York: WH Freeman.
Branden C & Tooze J (1999) Introduction to Protein Structure, 2nd ed. New York: Garland Science.
Bretscher MS (1985) The molecules of the cell membrane. *Sci Am* 253:100–109.
Burley SK & Petsko GA (1988) Weakly polar interactions in proteins. *Adv Protein Chem* 39:125–189.
De Duve C (2005) Singularities: Landmarks on the Pathways of Life. Cambridge: Cambridge University Press.
Dowhan W (1997) Molecular basis for membrane phospholipid diversity: Why are there so many lipids? *Annu Rev Biochem* 66:199–232.
Eisenberg D & Kauzman W (1969) The Structure and Properties of Water. Oxford: Oxford University Press.
Fersht AR (1987) The hydrogen bond in molecular recognition. *Trends Biochem Sci* 12:301–304.
Franks F (1993) Water. Cambridge: Royal Society of Chemistry.
Henderson LJ (1927) The Fitness of the Environment, 1958 ed. Boston: Beacon.
Neidhardt FC, Ingraham JL & Schaechter M (1990) Physiology of the Bacterial Cell: A Molecular Approach. Sunderland, MA: Sinauer.
Pauling L (1960) The Nature of the Chemical Bond, 3rd ed. Ithaca, NY: Cornell University Press.
Saenger W (1984) Principles of Nucleic Acid Structure. New York: Springer.
Sharon N (1980) Carbohydrates. *Sci Am* 243:90–116.
Stillinger FH (1980) Water revisited. *Science* 209:451–457.
Tanford C (1978) The hydrophobic effect and the organization of living matter. *Science* 200:1012–1018.
Tanford C (1980) The Hydrophobic Effect. Formation of Micelles and Biological Membranes, 2nd ed. New York: John Wiley.

Catalysis and the Use of Energy by Cells

Atkins PW (1994) The Second Law: Energy, Chaos and Form. New York: Scientific American Books.
Atkins PW & De Paula JD (2006) Physical Chemistry for the Life Sciences. Oxford: Oxford University Press.
Baldwin JE & Krebs H (1981) The Evolution of Metabolic Cycles. *Nature* 291:381–382.
Berg HC (1983) Random Walks in Biology. Princeton, NJ: Princeton University Press.
Dickerson RE (1969) Molecular Thermodynamics. Menlo Park, CA: Benjamin Cummings.
Dill KA & Bromberg S (2003) Molecular Driving Forces: Statistical Thermodynamics in Chemistry and Biology. New York: Garland Science.
Dressler D & Potter H (1991) Discovering Enzymes. New York: Scientific American Library.
Einstein A (1956) Investigations on the Theory of Brownian Movement. New York: Dover.
Fruton JS (1999) Proteins, Enzymes, Genes: The Interplay of Chemistry and Biology. New Haven: Yale University Press.
Goodsell DS (1991) Inside a living cell. *Trends Biochem Sci* 16:203–206.
Karplus M & McCammon JA (1986) The dynamics of proteins. *Sci Am* 254:42–51.
Karplus M & Petsko GA (1990) Molecular dynamics simulations in biology. *Nature* 347:631–639.
Kauzmann W (1967) Thermodynamics and Statistics: with Applications to Gases. In Thermal Properties of Matter Vol 2. New York: WA Benjamin, Inc.
Kornberg A (1989) For the Love of Enzymes. Cambridge, MA: Harvard University Press.
Lavenda BH (1985) Brownian Motion. *Sci Am* 252:70–85.
Lawlor DW (2001) Photosynthesis, 3rd ed. Oxford: BIOS.
Lehninger AL (1971) The Molecular Basis of Biological Energy Transformations, 2nd ed. Menlo Park, CA: Benjamin Cummings.
Lipmann F (1941) Metabolic generation and utilization of phosphate bond energy. *Adv Enzymol* 1:99–162.
Lipmann F (1971) Wanderings of a Biochemist. New York: Wiley.
Nisbet EE & Sleep NH (2001) The habitat and nature of early life. *Nature* 409:1083–1091.
Racker E (1980) From Pasteur to Mitchell: a hundred years of bioenergetics. *Fed Proc* 39:210–215.
Schrödinger E (1944 & 1958) What is Life?: The Physical Aspect of the Living Cell and Mind and Matter, 1992 combined ed. Cambridge: Cambridge University Press.
van Holde KE, Johnson WC & Ho PS (2005) Principles of Physical Biochemistry, 2nd ed. Upper Saddle River, NJ: Prentice Hall.
Walsh C (2001) Enabling the chemistry of life. *Nature* 409:226–231.
Westheimer FH (1987) Why nature chose phosphates. *Science* 235:1173–1178.
Youvan DC & Marrs BL (1987) Molecular mechanisms of photosynthesis. *Sci Am* 256:42–49.

How Cells Obtain Energy from Food

Cramer WA & Knaff DB (1990) Energy Transduction in Biological Membranes. New York: Springer-Verlag.
Dismukes GC, Klimov VV, Baranov SV et al (2001) The origin of atmospheric oxygen on Earth: The innovation of oxygenic photosyntheis. *Proc Nat Acad Sci USA* 98:2170–2175.
Fell D (1997) Understanding the Control of Metabolism. London: Portland Press.
Flatt JP (1995) Use and storage of carbohydrate and fat. *Am J Clin Nutr* 61, 952S–959S.
Friedmann HC (2004) From *Butybacterium* to *E. coli*: An essay on unity in biochemistry. *Perspect Biol Med* 47:47–66.
Fothergill-Gilmore LA (1986) The evolution of the glycolytic pathway. *Trends Biochem Sci* 11:47–51.
Heinrich R, Melendez-Hevia E, Montero F et al (1999) The structural design of glycolysis: An evolutionary approach. *Biochem Soc Trans* 27:294–298.
Huynen MA, Dandekar T & Bork P (1999) Variation and evolution of the citric-acid cycle: a genomic perspective. *Trends Microbiol* 7:281–291.
Kornberg HL (2000) Krebs and his trinity of cycles. *Nature Rev Mol Cell Biol* 1:225–228.
Krebs HA & Martin A (1981) Reminiscences and Reflections. Oxford/New York: Clarendon Press/Oxford University Press.
Krebs HA (1970) The history of the tricarboxylic acid cycle. *Perspect Biol Med* 14:154–170.
Martin BR (1987) Metabolic Regulation: A Molecular Approach. Oxford: Blackwell Scientific.
McGilvery RW (1983) Biochemistry: A Functional Approach, 3rd ed. Philadelphia: Saunders.
Morowitz HJ (1993) Beginnings of Cellular Life: Metabolism Recapitulates Biogenesis. New Haven: Yale University Press
Newsholme EA & Stark C (1973) Regulation of Metabolism. New York: Wiley.

3

Proteins

When we look at a cell through a microscope or analyze its electrical or biochemical activity, we are, in essence, observing proteins. Proteins constitute most of a cell's dry mass. They are not only the cell's building blocks; they also execute nearly all the cell's functions. Thus, enzymes provide the intricate molecular surfaces in a cell that promote its many chemical reactions. Proteins embedded in the plasma membrane form channels and pumps that control the passage of small molecules into and out of the cell. Other proteins carry messages from one cell to another, or act as signal integrators that relay sets of signals inward from the plasma membrane to the cell nucleus. Yet others serve as tiny molecular machines with moving parts: *kinesin*, for example, propels organelles through the cytoplasm; *topoisomerase* can untangle knotted DNA molecules. Other specialized proteins act as antibodies, toxins, hormones, antifreeze molecules, elastic fibers, ropes, or sources of luminescence. Before we can hope to understand how genes work, how muscles contract, how nerves conduct electricity, how embryos develop, or how our bodies function, we must attain a deep understanding of proteins.

In This Chapter

THE SHAPE AND STRUCTURE OF PROTEINS 125

PROTEIN FUNCTION 152

THE SHAPE AND STRUCTURE OF PROTEINS

From a chemical point of view, proteins are by far the most structurally complex and functionally sophisticated molecules known. This is perhaps not surprising, once we realize that the structure and chemistry of each protein has been developed and fine-tuned over billions of years of evolutionary history. Yet, even to experts, the remarkable versatility of proteins can seem truly amazing.

In this section, we consider how the location of each amino acid in the long string of amino acids that forms a protein determines its three-dimensional shape. Later in the chapter, we use this understanding of protein structure at the atomic level to describe how the precise shape of each protein molecule determines its function in a cell.

The Shape of a Protein Is Specified by Its Amino Acid Sequence

There are 20 types of amino acids in proteins, each with different chemical properties. A **protein** molecule is made from a long chain of these amino acids, each linked to its neighbor through a covalent peptide bond. Proteins are therefore also known as *polypeptides*. Each type of protein has a unique sequence of amino acids, and there are many thousands of different proteins, each with its own particular amino acid sequence.

The repeating sequence of atoms along the core of the polypeptide chain is referred to as the **polypeptide backbone**. Attached to this repetitive chain are those portions of the amino acids that are not involved in making a peptide bond and that give each amino acid its unique properties: the 20 different amino acid **side chains** (**Figure 3–1**). Some of these side chains are nonpolar and hydrophobic ("water-fearing"), others are negatively or positively charged, some readily form covalent bonds, and so on. **Panel 3–1** (pp. 128–129) shows their atomic structures and **Figure 3–2** lists their abbreviations.

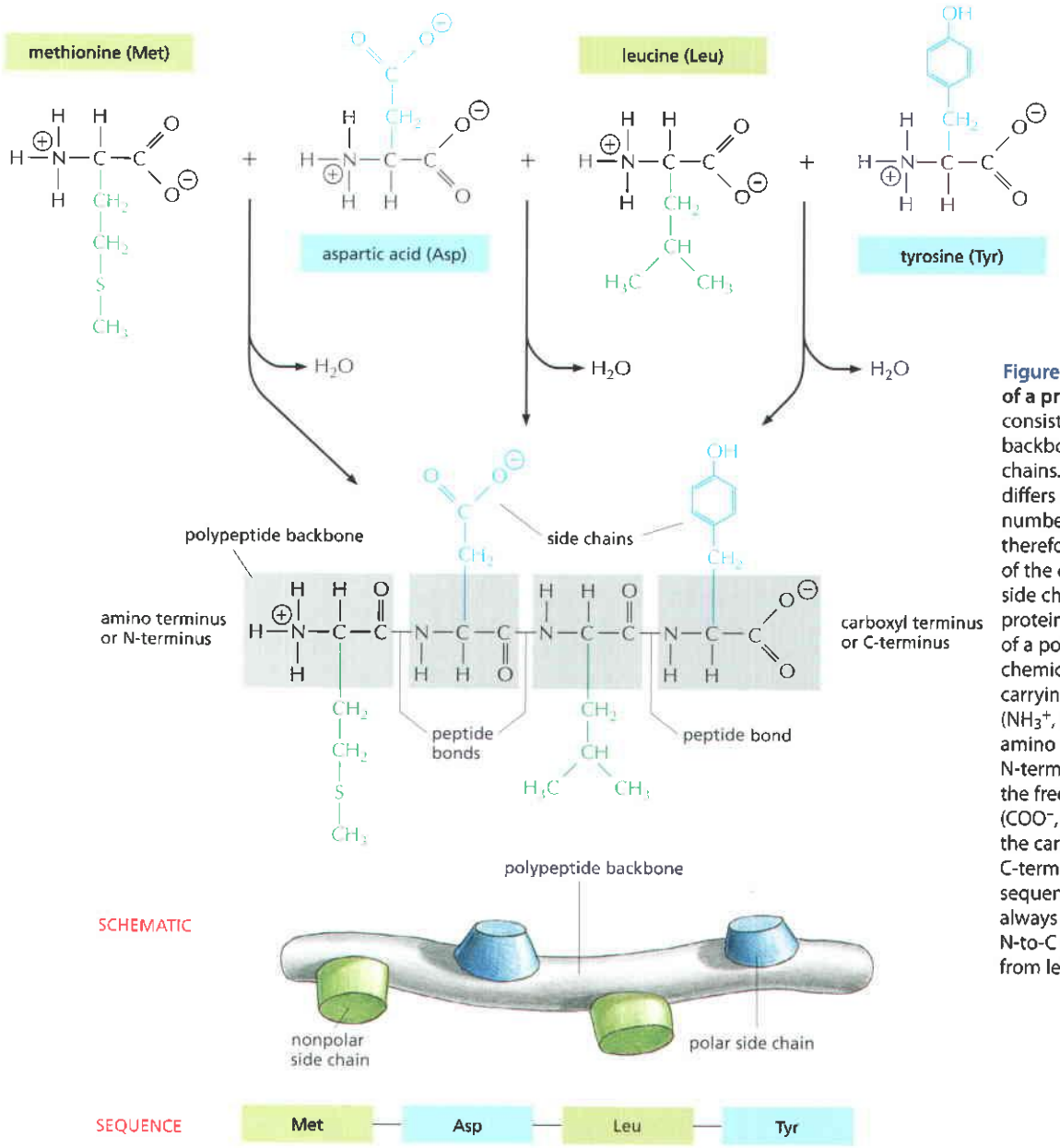


Figure 3–1 The components of a protein. A protein consists of a polypeptide backbone with attached side chains. Each type of protein differs in its sequence and number of amino acids; therefore, it is the sequence of the chemically different side chains that makes each protein distinct. The two ends of a polypeptide chain are chemically different: the end carrying the free amino group (NH_3^+, also written NH_2) is the amino terminus, or N-terminus, and that carrying the free carboxyl group (COO^-, also written COOH) is the carboxyl terminus or C-terminus. The amino acid sequence of a protein is always presented in the N-to-C direction, reading from left to right.

As discussed in Chapter 2, atoms behave almost as if they were hard spheres with a definite radius (their *van der Waals radius*). The requirement that no two atoms overlap limits greatly the possible bond angles in a polypeptide chain (**Figure 3–3**). This constraint and other steric interactions severely restrict the possible three-dimensional arrangements of atoms (or *conformations*). Nevertheless, a long flexible chain, such as a protein, can still fold in an enormous number of ways.

The folding of a protein chain is, however, further constrained by many different sets of weak *noncovalent bonds* that form between one part of the chain and another. These involve atoms in the polypeptide backbone, as well as atoms in the amino acid side chains. There are three types of weak bonds: *hydrogen bonds*, *electrostatic attractions*, and *van der Waals attractions*, as explained in Chapter 2 (see p. 54). Individual noncovalent bonds are 30–300 times weaker than the typical covalent bonds that create biological molecules. But many weak bonds acting in parallel can hold two regions of a polypeptide chain tightly together. In this way, the combined strength of large numbers of such noncovalent bonds determines the stability of each folded shape (**Figure 3–4**).

AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negative
Glutamic acid	Glu	E	negative
Arginine	Arg	R	positive
Lysine	Lys	K	positive
Histidine	His	H	positive
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

POLAR AMINO ACIDS

AMINO ACID			SIDE CHAIN
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	F	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

NONPOLAR AMINO ACIDS

Figure 3–2 The 20 amino acids found in proteins. Each amino acid has a three-letter and a one-letter abbreviation. There are equal numbers of polar and nonpolar side chains; however, some side chains listed here as polar are large enough to have some non-polar properties (for example, Tyr, Thr, Arg, Lys). For atomic structures, see Panel 3–1 (pp. 128–129).

A fourth weak force also has a central role in determining the shape of a protein. As described in Chapter 2, hydrophobic molecules, including the nonpolar side chains of particular amino acids, tend to be forced together in an aqueous environment in order to minimize their disruptive effect on the hydrogen-bonded network of water molecules (see p. 54 and Panel 2–2, pp. 108–109). Therefore, an important factor governing the folding of any protein is the distribution of its polar and nonpolar amino acids. The nonpolar (hydrophobic) side chains in a protein—belonging to such amino acids as phenylalanine, leucine, valine, and tryptophan—tend to cluster in the interior of the molecule (just as hydrophobic oil droplets coalesce in water to form one large droplet). This enables them to avoid contact with the water that surrounds them inside a cell. In contrast, polar groups—such as those belonging to arginine, glutamine, and histidine—tend to arrange themselves near the outside of the molecule, where they can form hydrogen bonds with water and with other polar molecules (**Figure 3–5**). Polar amino acids buried within the protein are usually hydrogen-bonded to other polar amino acids or to the polypeptide backbone.

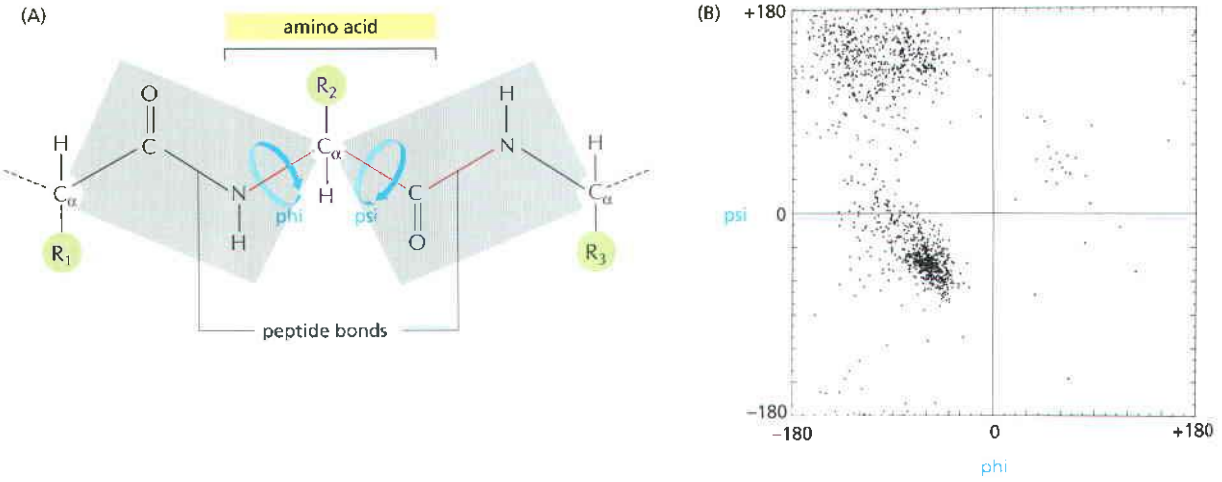
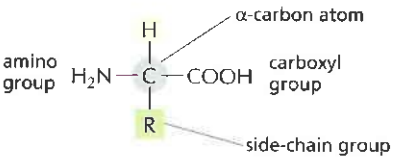


Figure 3–3 Steric limitations on the bond angles in a polypeptide chain. (A) Each amino acid contributes three bonds *(red)* to the backbone of the chain. The peptide bond is planar *(gray shading)* and does not permit rotation. By contrast, rotation can occur about the C_α–C bond, whose angle of rotation is called psi (ψ), and about the N–C_α bond, whose angle of rotation is called phi (ϕ). By convention, an R group is often used to denote an amino acid side chain *(green circles)*. (B) The conformation of the main-chain atoms in a protein is determined by one pair of ϕ and ψ angles for each amino acid; because of steric collisions between atoms within each amino acid, most pairs of ϕ and ψ angles do not occur. In this so-called Ramachandran plot, each dot represents an observed pair of angles in a protein. The cluster of dots in the bottom left quadrant represents all of the amino acids that are located in α-helix structures (see Figure 3–7A). (B, from J. Richardson, *Adv. Prot. Chem.* 34:174–175, 1981. With permission from Academic Press.)

THE AMINO ACID

The general formula of an amino acid is

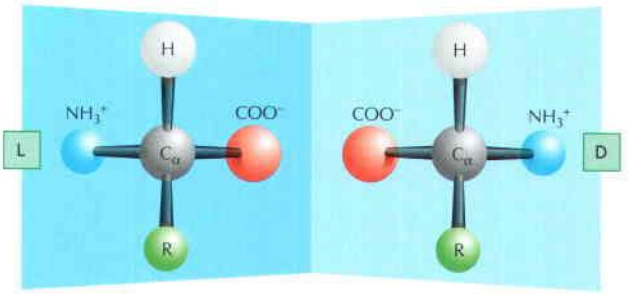


R is commonly one of 20 different side chains. At pH 7 both the amino and carboxyl groups are ionized.



OPTICAL ISOMERS

The α-carbon atom is asymmetric, which allows for two mirror image (or stereo-) isomers, L and D.



Proteins consist exclusively of L-amino acids.

FAMILIES OF AMINO ACIDS

The common amino acids are grouped according to whether their side chains are

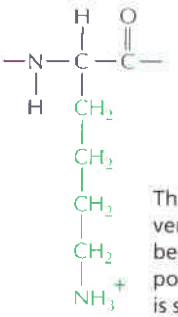
- acidic
- basic
- uncharged polar
- nonpolar

These 20 amino acids are given both three-letter and one-letter abbreviations.

Thus: alanine = Ala = A

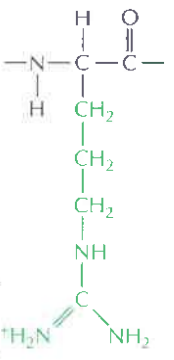
BASIC SIDE CHAINS

lysine (Lys, or K)

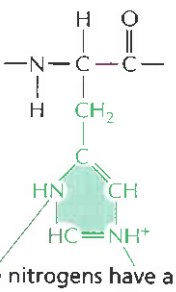


This group is very basic because its positive charge is stabilized by resonance.

arginine (Arg, or R)



histidine (His, or H)

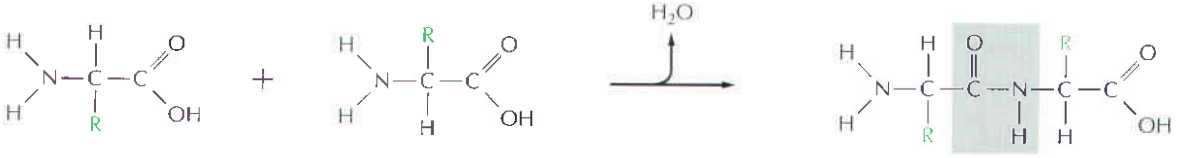


These nitrogens have a relatively weak affinity for an H^+ and are only partly positive at neutral pH.

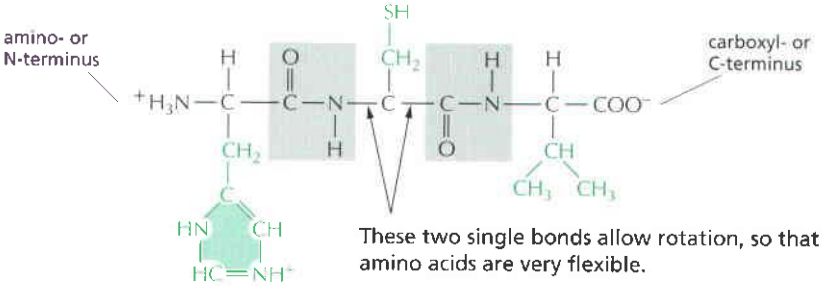
PEPTIDE BONDS

Amino acids are commonly joined together by an amide linkage, called a peptide bond.

Peptide bond: The four atoms in each *gray box* form a rigid planar unit. There is no rotation around the C–N bond.



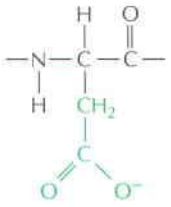
Proteins are long polymers of amino acids linked by peptide bonds, and they are always written with the N-terminus toward the left. The sequence of this tripeptide is histidine-cysteine-valine.



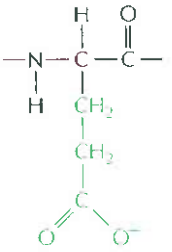
These two single bonds allow rotation, so that long chains of amino acids are very flexible.

ACIDIC SIDE CHAINS

aspartic acid
(Asp, or D)

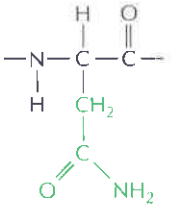


glutamic acid
(Glu, or E)

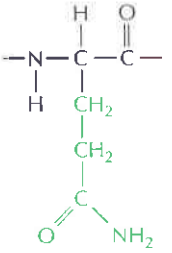


UNCHARGED POLAR SIDE CHAINS

asparagine
(Asn, or N)

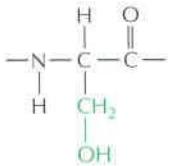


glutamine
(Gln, or Q)

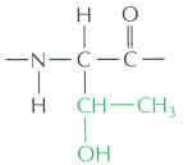


Although the amide N is not charged at neutral pH, it is polar.

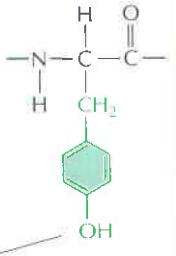
serine
(Ser, or S)



threonine
(Thr, or T)



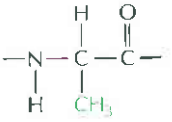
tyrosine
(Tyr, or Y)



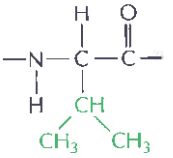
The –OH group is polar.

NONPOLAR SIDE CHAINS

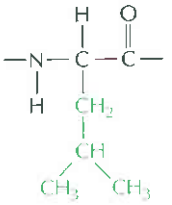
alanine
(Ala, or A)



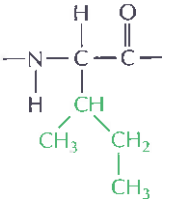
valine
(Val, or V)



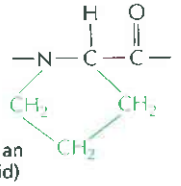
leucine
(Leu, or L)



isoleucine
(Ile, or I)

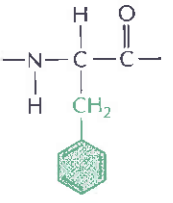


proline
(Pro, or P)

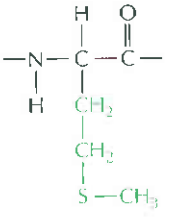


(actually an imino acid)

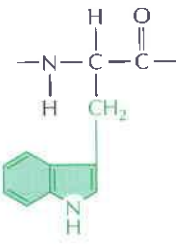
phenylalanine
(Phe, or F)



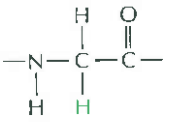
methionine
(Met, or M)



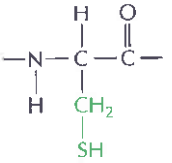
tryptophan
(Trp, or W)



glycine
(Gly, or G)



cysteine
(Cys, or C)



Disulfide bonds can form between two cysteine side chains in proteins.



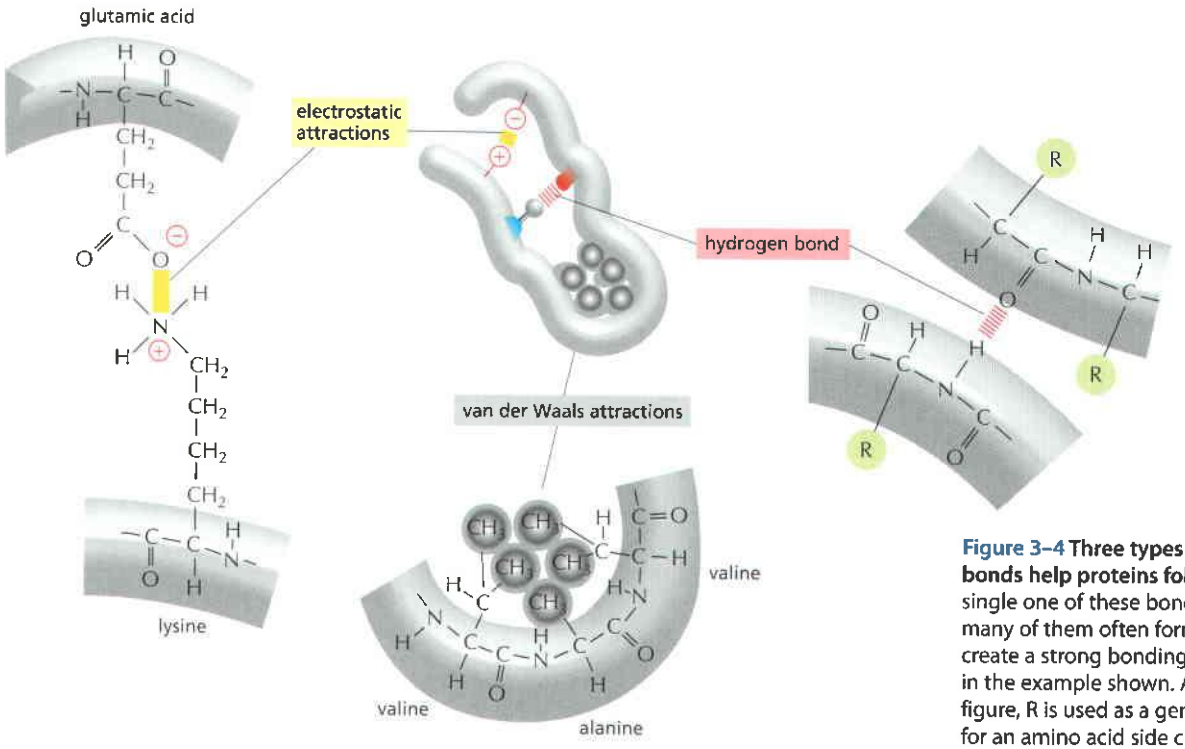


Figure 3–4 Three types of noncovalent bonds help proteins fold. Although a single one of these bonds is quite weak, many of them often form together to create a strong bonding arrangement, as in the example shown. As in the previous figure, R is used as a general designation for an amino acid side chain.

Proteins Fold into a Conformation of Lowest Energy

As a result of all of these interactions, most proteins have a particular three-dimensional structure, which is determined by the order of the amino acids in its chain. The final folded structure, or **conformation**, of any polypeptide chain is generally the one that minimizes its free energy. Biologists have studied protein folding in a test tube by using highly purified proteins. Treatment with certain solvents, which disrupt the noncovalent interactions holding the folded chain together, unfolds, or *denatures*, a protein. This treatment converts the protein into a flexible polypeptide chain that has lost its natural shape. When the denaturing solvent is removed, the protein often refolds spontaneously, or *renatures*, into its original conformation (**Figure 3–6**). This indicates that the amino acid sequence contains all the information needed for specifying the three-dimensional shape of a protein, which is a critical point for understanding cell function.

Each protein normally folds up into a single stable conformation. However, the conformation changes slightly when the protein interacts with other molecules in the cell. This change in shape is often crucial to the function of the protein, as we see later.

Although a protein chain can fold into its correct conformation without outside help, in a living cell special proteins called *molecular chaperones* often assist in protein folding. Molecular chaperones bind to partly folded polypeptide chains and help them progress along the most energetically favorable folding

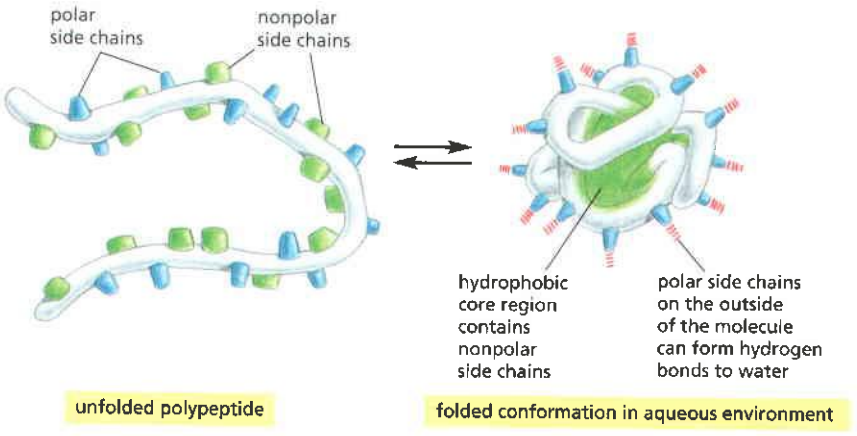


Figure 3–5 How a protein folds into a compact conformation. The polar amino acid side chains tend to gather on the outside of the protein, where they can interact with water; the nonpolar amino acid side chains are buried on the inside to form a tightly packed hydrophobic core of atoms that are hidden from water. In this schematic drawing, the protein contains only about 30 amino acids.

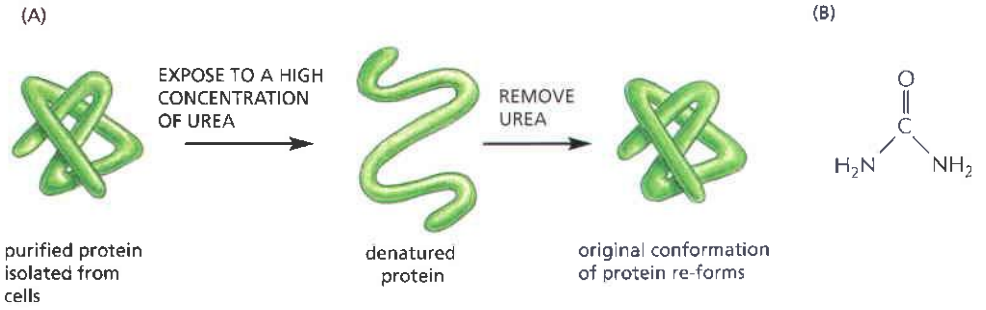


Figure 3–6 The refolding of a denatured protein. (A) This type of experiment, first performed more than 40 years ago, demonstrates that a protein's conformation is determined solely by its amino acid sequence. (B) The structure of urea. Urea is very soluble in water and unfolds proteins at high concentrations, where there is about one urea molecule for every six water molecules.

pathway. In the crowded conditions of the cytoplasm, chaperones prevent the temporarily exposed hydrophobic regions in newly synthesized protein chains from associating with each other to form protein aggregates (see p. 388). However, the final three-dimensional shape of the protein is still specified by its amino acid sequence: chaperones simply make the folding process more reliable.

Proteins come in a wide variety of shapes, and they are generally between 50 and 2000 amino acids long. Large proteins usually consist of several distinct *protein domains*—structural units that fold more or less independently of each other, as we discuss below. Since the detailed structure of any protein is complicated, several different representations are used to depict the protein's structure, each emphasizing different features.

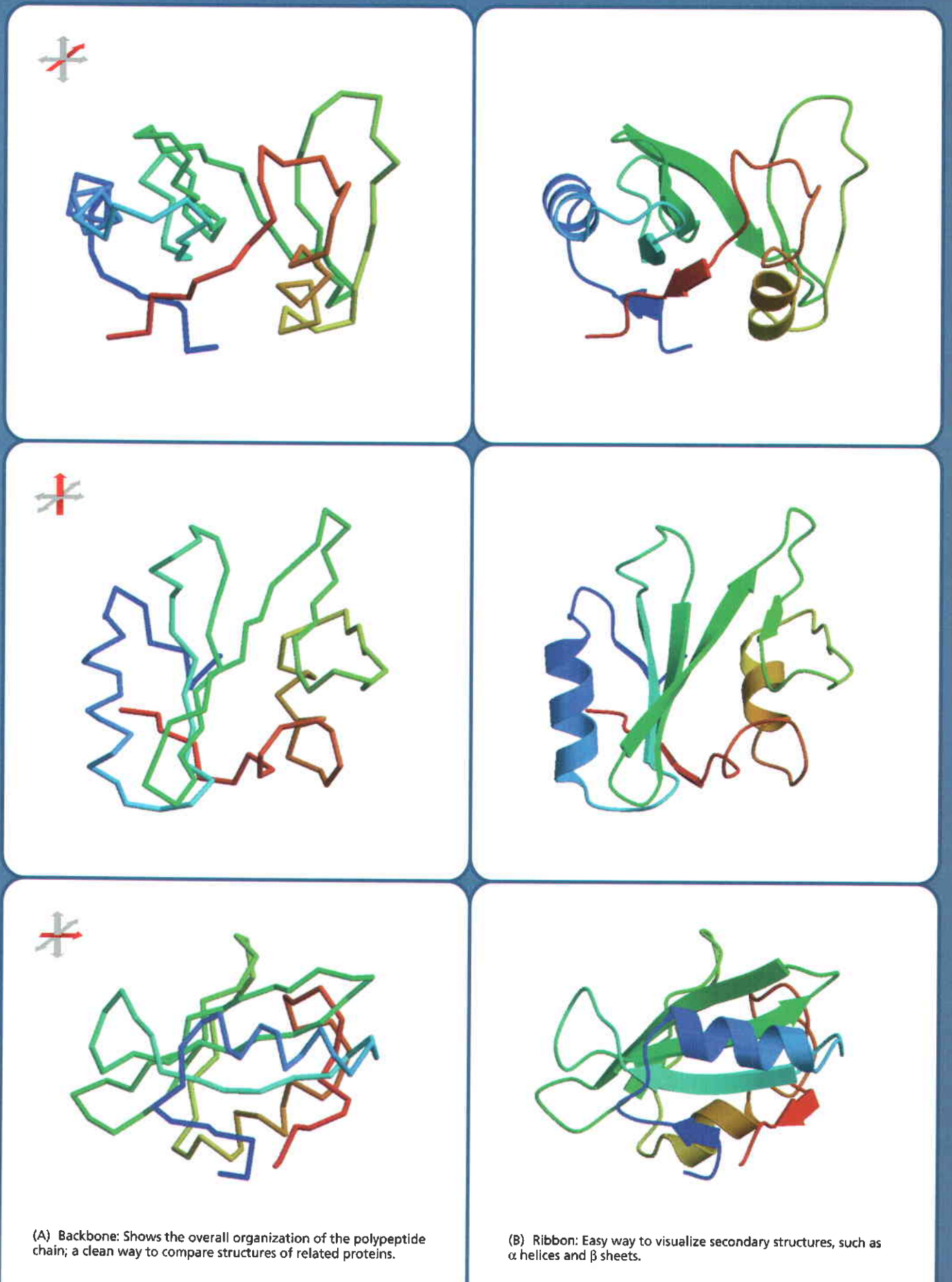
Panel 3–2 (pp. 132–133) presents four different representations of a protein domain called SH2, which has important functions in eucaryotic cells. Constructed from a string of 100 amino acids, the structure is displayed as (A) a polypeptide backbone model, (B) a ribbon model, (C) a wire model that includes the amino acid side chains, and (D) a space-filling model. Each of the three horizontal rows shows the protein in a different orientation, and the image is colored in a way that allows the polypeptide chain to be followed from its N-terminus *(purple)* to its C-terminus *(red)*. <GTGA>

Panel 3–2 shows that a protein's conformation is amazingly complex, even for a structure as small as the SH2 domain. But the description of protein structures can be simplified because they are built up from combinations of several common structural motifs, as we discuss next.

The α Helix and the β Sheet Are Common Folding Patterns

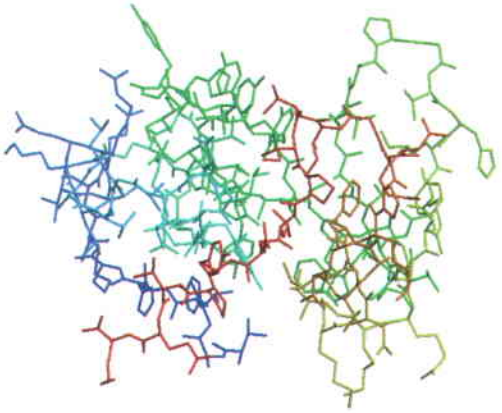
When we compare the three-dimensional structures of many different protein molecules, it becomes clear that, although the overall conformation of each protein is unique, two regular folding patterns are often found in parts of them. Both patterns were discovered more than 50 years ago from studies of hair and silk. The first folding pattern to be discovered, called the **α helix**, was found in the protein *α-keratin*, which is abundant in skin and its derivatives—such as hair, nails, and horns. Within a year of the discovery of the α helix, a second folded structure, called a **β sheet**, was found in the protein *fibroin*, the major constituent of silk. These two patterns are particularly common because they result from hydrogen-bonding between the N–H and C=O groups in the polypeptide backbone, without involving the side chains of the amino acids. Thus, many different amino acid sequences can form them. In each case, the protein chain adopts a regular, repeating conformation. **Figure 3–7** shows these two conformations, as well as the abbreviations that are used to denote them in ribbon models of proteins.

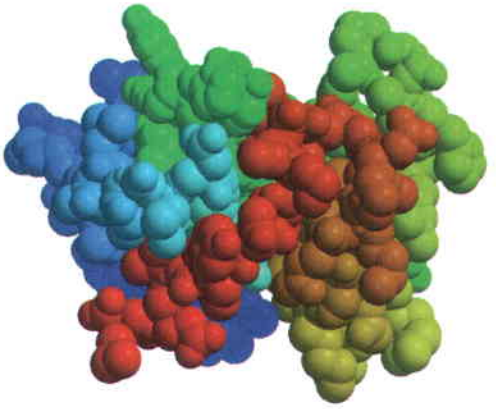
The core of many proteins contains extensive regions of β sheet. As shown in **Figure 3–8**, these β sheets can form either from neighboring polypeptide chains that run in the same orientation (parallel chains) or from a polypeptide chain that folds back and forth upon itself, with each section of the chain running in the direction opposite to that of its immediate neighbors (antiparallel chains). Both types of β sheet produce a very rigid structure, held together by hydrogen bonds that connect the peptide bonds in neighboring chains (see Figure 3–7D).

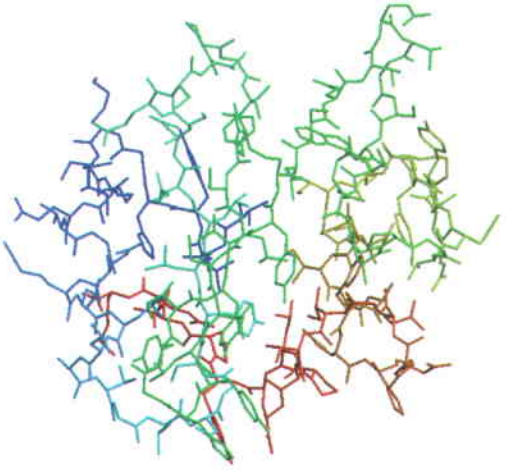


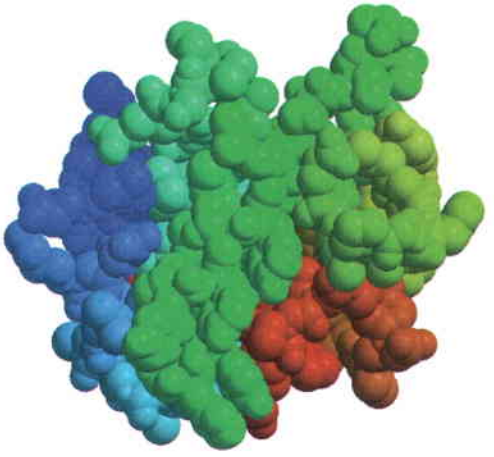
(A) Backbone: Shows the overall organization of the polypeptide chain; a clean way to compare structures of related proteins.

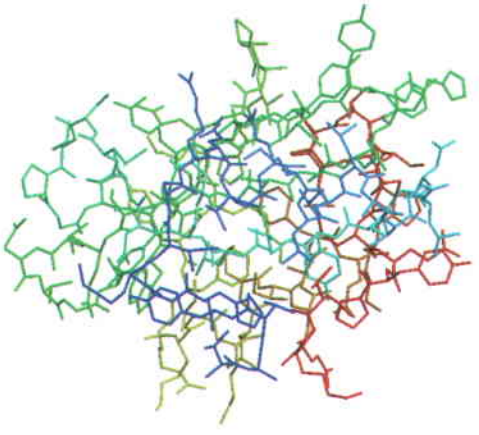
(B) Ribbon: Easy way to visualize secondary structures, such as α helices and β sheets.



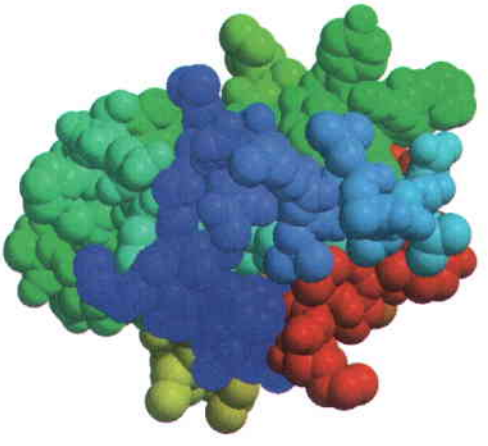








(C) Wire: Highlights side chains and their relative proximities; useful for predicting which amino acids might be involved in a protein's activity, particularly if the protein is an enzyme.



(D) Space-filling: Provides contour map of the protein; gives a feel for the shape of the protein and shows which amino acid side chains are exposed on its surface. Shows how the protein might look to a small molecule, such as water, or to another protein.

(Courtesy of David Lawson.)

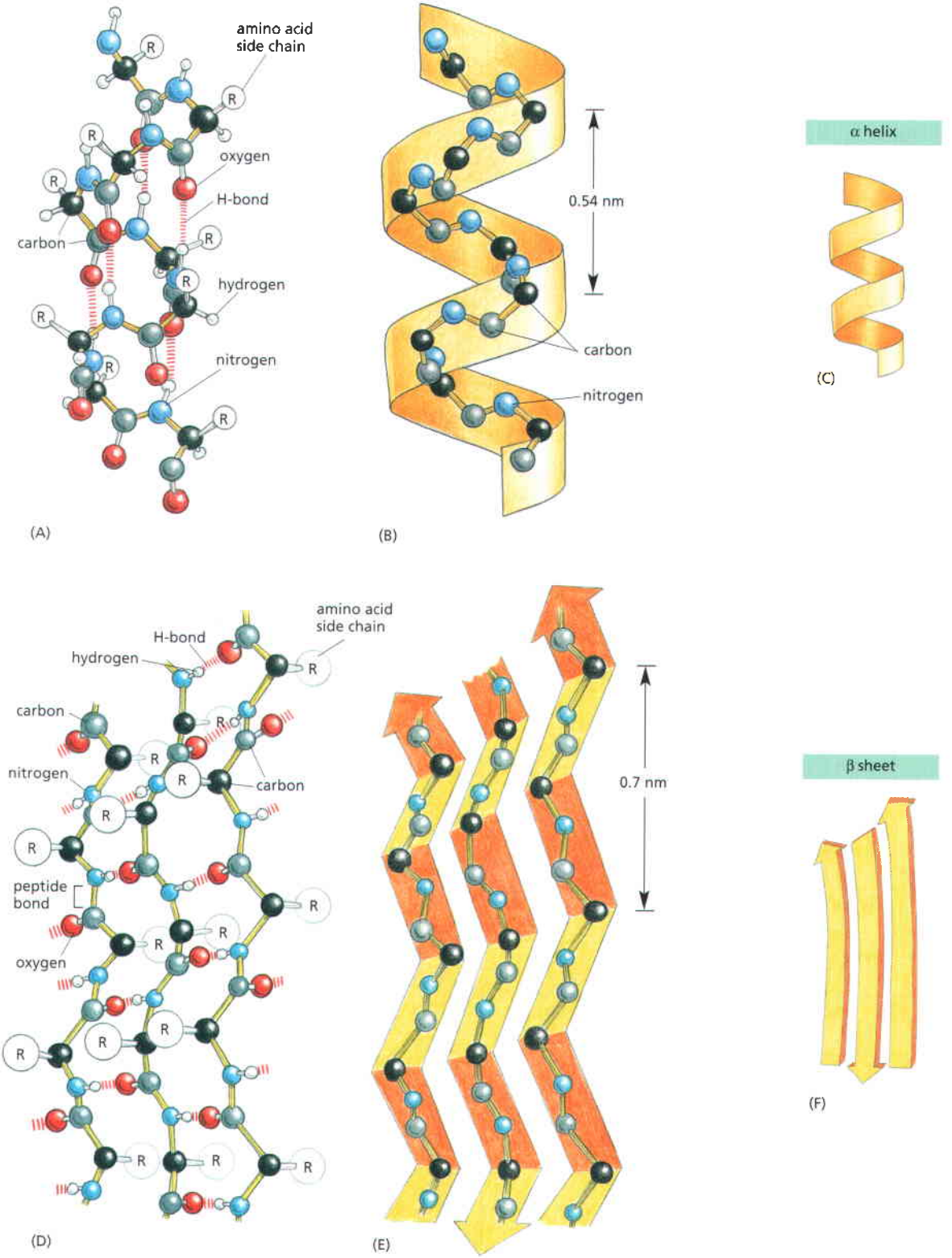


Figure 3–7 The regular conformation of the polypeptide backbone in the α helix and the β sheet. <GTAG> <TGCT> (A, B, and C) The α helix. The N–H of every peptide bond is hydrogen-bonded to the C=O of a neighboring peptide bond located four peptide bonds away in the same chain. Note that all of the N–H groups point up in this diagram and that all of the C=O groups point down (toward the C-terminus); this gives a polarity to the helix, with the C-terminus having a partial negative and the N-terminus a partial positive charge. (D, E, and F) The β sheet. In this example, adjacent peptide chains run in opposite (antiparallel) directions. Hydrogen-bonding between peptide bonds in different strands holds the individual polypeptide chains (strands) together in a β sheet, and the amino acid side chains in each strand alternately project above and below the plane of the sheet. (A) and (D) show all the atoms in the polypeptide backbone, but the amino acid side chains are truncated and denoted by R. In contrast, (B) and (E) show the backbone atoms only, while (C) and (F) display the shorthand symbols that are used to represent the α helix and the β sheet in ribbon drawings of proteins (see Panel 3–2B).

An α helix is generated when a single polypeptide chain twists around on itself to form a rigid cylinder. A hydrogen bond forms between every fourth peptide bond, linking the C=O of one peptide bond to the N–H of another (see Figure 3–7A). This gives rise to a regular helix with a complete turn every 3.6 amino acids. Note that the protein domain illustrated in Panel 3–2 contains two α helices, as well as a three-stranded antiparallel β sheet.

Regions of α helix are especially abundant in proteins located in cell membranes, such as transport proteins and receptors. As we discuss in Chapter 10, those portions of a transmembrane protein that cross the lipid bilayer usually cross as an α helix composed largely of amino acids with nonpolar side chains. The polypeptide backbone, which is hydrophilic, is hydrogen-bonded to itself in the α helix and shielded from the hydrophobic lipid environment of the membrane by its protruding nonpolar side chains (see also Figure 3–78).

In other proteins, α helices wrap around each other to form a particularly stable structure, known as a **coiled-coil**. This structure can form when the two (or in some cases three) α helices have most of their nonpolar (hydrophobic) side chains on one side, so that they can twist around each other with these side chains facing inward (**Figure 3–9**). Long rodlike coiled-coils provide the structural framework for many elongated proteins. Examples are α-keratin, which forms the intracellular fibers that reinforce the outer layer of the skin and its appendages, and the myosin molecules responsible for muscle contraction.

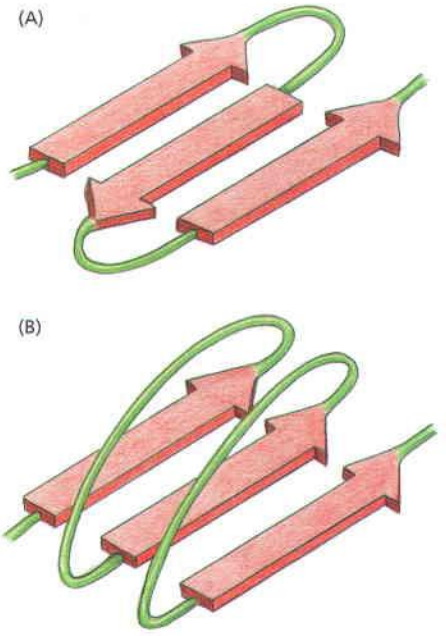


Figure 3–8 Two types of β sheet structures. (A) An antiparallel β sheet (see Figure 3–7D). (B) A parallel β sheet. Both of these structures are common in proteins.

Protein Domains Are Modular Units from which Larger Proteins Are Built

Even a small protein molecule is built from thousands of atoms linked together by precisely oriented covalent and noncovalent bonds, and it is extremely difficult to visualize such a complicated structure without a three-dimensional display. For

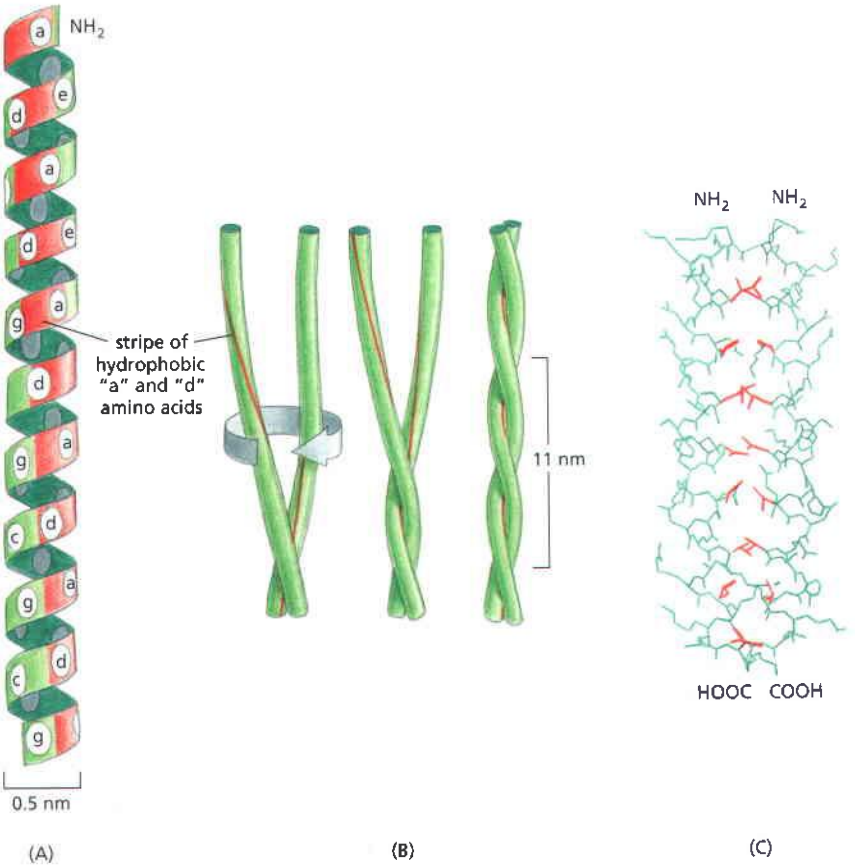


Figure 3–9 A coiled-coil. <CGGA>
(A) A single α helix, with successive amino acid side chains labeled in a sevenfold sequence, "abcdefg" (*from bottom to top*). Amino acids "a" and "d" in such a sequence lie close together on the cylinder surface, forming a "stripe" (*red*) that winds slowly around the α helix. Proteins that form coiled-coils typically have nonpolar amino acids at positions "a" and "d." Consequently, as shown in (B), the two α helices can wrap around each other with the nonpolar side chains of one α helix interacting with the nonpolar side chains of the other, while the more hydrophilic amino acid side chains are left exposed to the aqueous environment. (C) The atomic structure of a coiled-coil determined by x-ray crystallography. The *red* side chains are nonpolar.

this reason, biologists use various graphic and computer-based aids. A DVD that accompanies this book contains computer-generated images of selected proteins, displayed and rotated on the screen in a variety of formats.

Biologists distinguish four levels of organization in the structure of a protein. The amino acid sequence is known as the **primary structure**. Stretches of polypeptide chain that form α helices and β sheets constitute the protein's **secondary structure**. The full three-dimensional organization of a polypeptide chain is sometimes referred to as the **tertiary structure**, and if a particular protein molecule is formed as a complex of more than one polypeptide chain, the complete structure is designated as the **quaternary structure**.

Studies of the conformation, function, and evolution of proteins have also revealed the central importance of a unit of organization distinct from these four. This is the **protein domain**, a substructure produced by any part of a polypeptide chain that can fold independently into a compact, stable structure. A domain usually contains between 40 and 350 amino acids, and it is the modular unit from which many larger proteins are constructed.

The different domains of a protein are often associated with different functions. Figure 3–10 shows an example—the Src protein kinase, which functions in signaling pathways inside vertebrate cells (Src is pronounced "sarc"). This protein is considered to have three domains: the SH2 and SH3 domains have regulatory roles, while the C-terminal domain is responsible for the kinase catalytic activity. Later in the chapter, we shall return to this protein, in order to explain how proteins can form molecular switches that transmit information throughout cells.

Figure 3–11 presents ribbon models of three differently organized protein domains. As these examples illustrate, the polypeptide chain tends to cross the entire domain before making a sharp turn at the surface. The central core of a domain can be constructed from α helices, from β sheets, or from various combinations of these two fundamental folding elements. <CAGT>

The smallest protein molecules contain only a single domain, whereas larger proteins can contain as many as several dozen domains, often connected to each other by short, relatively unstructured lengths of polypeptide chain.

Few of the Many Possible Polypeptide Chains Will Be Useful to Cells

Since each of the 20 amino acids is chemically distinct and each can, in principle, occur at any position in a protein chain, there are $20 \times 20 \times 20 \times 20 = 160{,}000$ different possible polypeptide chains four amino acids long, or 20^n different possible polypeptide chains n amino acids long. For a typical protein length of

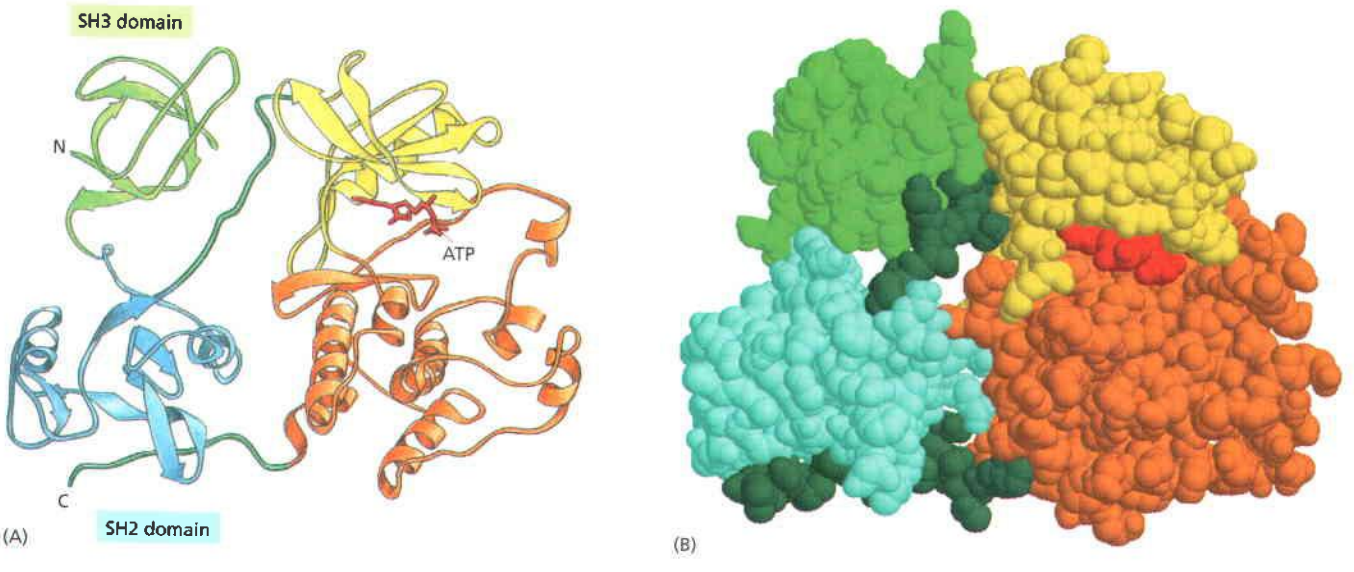


Figure 3–10 A protein formed from multiple domains. In the Src protein shown, a C-terminal domain with two lobes (*yellow* and *orange*) forms a protein kinase enzyme, while the SH2 and SH3 domains perform regulatory functions. (A) A ribbon model, with ATP substrate in *red*. (B) A spacing-filling model, with ATP substrate in *red*. Note that the site that binds ATP is positioned at the interface of the two lobes that form the kinase. The detailed structure of the SH2 domain is illustrated in Panel 3–2 (pp. 132–133).

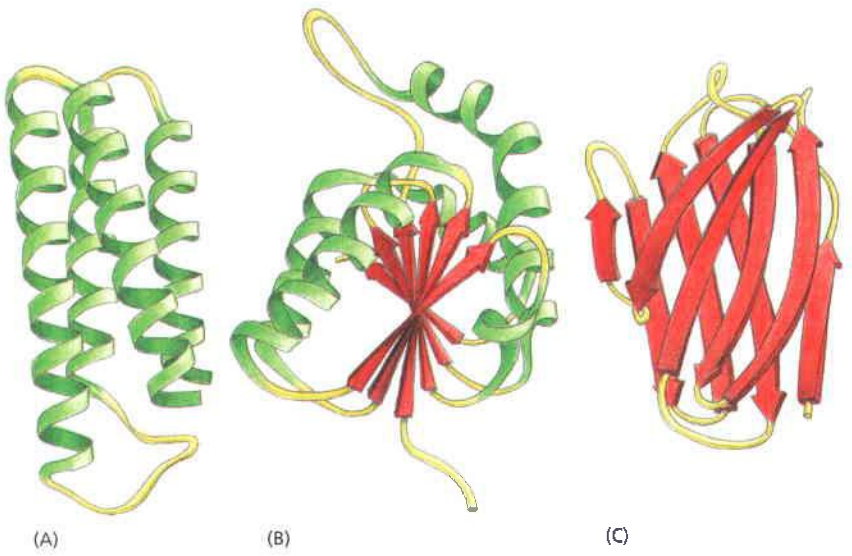


Figure 3–11 Ribbon models of three different protein domains. (A) Cytochrome b_{562}, a single-domain protein involved in electron transport in mitochondria. This protein is composed almost entirely of α helices. (B) The NAD-binding domain of the enzyme lactic dehydrogenase, which is composed of a mixture of α helices and parallel β sheets. (C) The variable domain of an immunoglobulin (antibody) light chain, composed of a sandwich of two antiparallel β sheets. In these examples, the α helices are shown in *green*, while strands organized as β sheets are denoted by *red arrows*.

Note how the polypeptide chain generally traverses back and forth across the entire domain, making sharp turns only at the protein surface. It is the protruding loop regions *(yellow)* that often form the binding sites for other molecules. (Adapted from drawings courtesy of Jane Richardson.)

about 300 amino acids, a cell could theoretically make more than 10^{390} (20^{300}) different polypeptide chains. This is such an enormous number that to produce just one molecule of each kind would require many more atoms than exist in the universe.

Only a very small fraction of this vast set of conceivable polypeptide chains would adopt a single, stable three-dimensional conformation—by some estimates, less than one in a billion. And yet the vast majority of proteins present in cells adopt unique and stable conformations. How is this possible? The answer lies in natural selection. A protein with an unpredictably variable structure and biochemical activity is unlikely to help the survival of a cell that contains it. Such proteins would therefore have been eliminated by natural selection through the enormously long trial-and-error process that underlies biological evolution.

Because evolution has selected for protein function in living organisms, the amino acid sequence of most present-day proteins is such that a single conformation is extremely stable. In addition, this conformation has its chemical properties finely tuned to enable the protein to perform a particular catalytic or structural function in the cell. Proteins are so precisely built that the change of even a few atoms in one amino acid can sometimes disrupt the structure of the whole molecule so severely that all function is lost.

Proteins Can Be Classified into Many Families

Once a protein had evolved that folded up into a stable conformation with useful properties, its structure could be modified during evolution to enable it to perform new functions. This process has been greatly accelerated by genetic mechanisms that occasionally duplicate genes, allowing one gene copy to evolve independently to perform a new function (discussed in Chapter 4). This type of event has occurred quite often in the past; as a result, many present-day proteins can be grouped into protein families, each family member having an amino acid sequence and a three-dimensional conformation that resemble those of the other family members.

Consider, for example, the *serine proteases*, a large family of protein-cleaving (proteolytic) enzymes that includes the digestive enzymes chymotrypsin, trypsin, and elastase, and several proteases involved in blood clotting. When the protease portions of any two of these enzymes are compared, parts of their amino acid sequences are found to match. The similarity of their three-dimensional conformations is even more striking: most of the detailed twists and turns in their polypeptide chains, which are several hundred amino acids long, are virtually identical (**Figure 3–12**). The many different serine proteases nevertheless

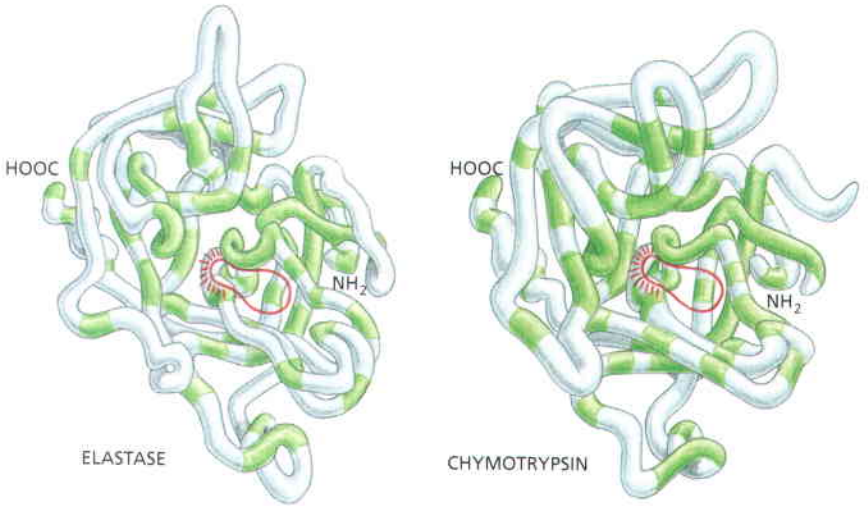


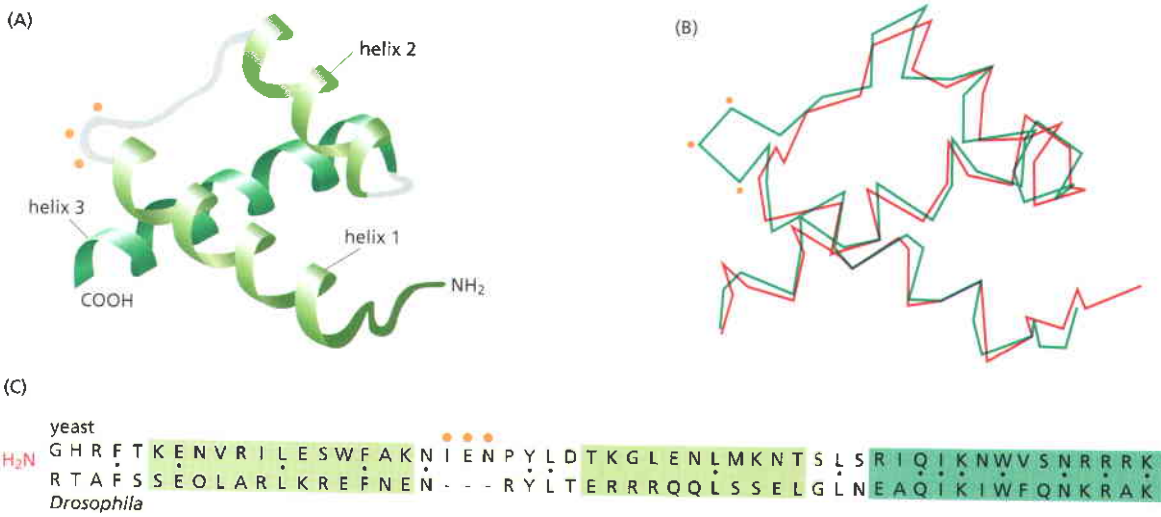
Figure 3–12 A comparison of the conformations of two serine proteases. The backbone conformations of elastase and chymotrypsin. Although only those amino acids in the polypeptide chain shaded in *green* are the same in the two proteins, the two conformations are very similar nearly everywhere. The active site of each enzyme is circled in *red;* this is where the peptide bonds of the proteins that serve as substrates are bound and cleaved by hydrolysis. The serine proteases derive their name from the amino acid serine, whose side chain is part of the active site of each enzyme and directly participates in the cleavage reaction.

have distinct enzymatic activities, each cleaving different proteins or the peptide bonds between different types of amino acids. Each therefore performs a distinct function in an organism.

The story we have told for the serine proteases could be repeated for hundreds of other protein families. In general, the structure of the different members of a protein family has been more highly conserved than has the amino acid sequence. In many cases, the amino acid sequences have diverged so far that we cannot be certain of a family relationship between two proteins without determining their three-dimensional structures. The yeast α2 protein and the *Drosophila* engrailed protein, for example, are both gene regulatory proteins in the homeodomain family (discussed in Chapter 7). Because they are identical in only 17 of their 60 amino acid residues, their relationship became certain only by comparing their three-dimensional structures (**Figure 3–13**). Many similar examples show that two proteins with more than 25% identity in their amino acid sequences usually share the same overall structure.

The various members of a large protein family often have distinct functions. Some of the amino acid changes that make family members different were no doubt selected in the course of evolution because they resulted in useful changes in biological activity, giving the individual family members the different functional properties they have today. But many other amino acid changes are effectively "neutral," having neither a beneficial nor a damaging effect on the basic structure and function of the protein. In addition, since mutation is a random process, there must also have been many deleterious changes that altered the three-dimensional structure of these proteins sufficiently to harm them.

Figure 3–13 A comparison of a class of DNA-binding domains, called homeodomains, in a pair of proteins from two organisms separated by more than a billion years of evolution. (A) A ribbon model of the structure common to both proteins. (B) A trace of the α-carbon positions. The three-dimensional structures shown were determined by x-ray crystallography for the yeast α2 protein *(green)* and the *Drosophila* engrailed protein *(red).* (C) A comparison of amino acid sequences for the region of the proteins shown in (A) and (B). *Black dots* mark sites with identical amino acids. *Orange dots* indicate the position of a three amino acid insert in the α2 protein. (Adapted from C. Wolberger et al., *Cell* 67:517–528, 1991. With permission from Elsevier.)



Such faulty proteins would have been lost whenever the individual organisms making them were at enough of a disadvantage to be eliminated by natural selection.

Protein families are readily recognized when the genome of any organism is sequenced; for example, the determination of the DNA sequence for the entire human genome has revealed that we contain about 24,000 protein-coding genes. Through sequence comparisons, we can assign the products of about 40 percent of these genes to known protein structures, belonging to more than 500 different protein families. Most of the proteins in each family have evolved to perform somewhat different functions, as for the enzymes elastase and chymotrypsin illustrated previously in Figure 3–12. These are sometimes called *paralogs* to distinguish them from the corresponding proteins in different organisms (*orthologs*, such as mouse and human elastase).

As described in Chapter 8, because of the powerful techniques of x-ray crystallography and nuclear magnetic resonance (NMR), we now know the three-dimensional shapes, or conformations, of more than 20,000 proteins. <GGCC> By carefully comparing the conformations of these proteins, structural biologists (that is, experts on the structure of biological molecules) have concluded that there are a limited number of ways in which protein domains fold up in nature—maybe as few as 2000. The structures for about 800 of these protein folds have thus far been determined. These known folds tend to be those most represented in the universe of protein structures: for example, 50 folds account for nearly three-fourths of the domain families with predicted structures. A complete catalog of the most significant protein folds that exist in living organisms would therefore seem to be within our reach.

Sequence Searches Can Identify Close Relatives

The present database of known protein sequences contains more than ten million entries, and it is growing very rapidly as more and more genomes are sequenced—revealing huge numbers of new genes that encode proteins. Powerful computer search programs are available that allow us to compare each newly discovered protein with this entire database, looking for possible relatives. Many proteins whose genes have evolved from a common ancestral gene can be identified by the discovery of statistically significant similarities in amino acid sequences.

With such a large number of proteins in the database, the search programs find many nonsignificant matches, resulting in a background noise level that makes it very difficult to pick out all but the closest relatives. Generally speaking, one requires a 30% identity in sequence to consider that two proteins match. However, we know the function of many short signature sequences ("fingerprints"), and these are widely used to find more distant relationships (**Figure 3–14**).

Protein comparisons are important because related structures often imply related functions. Many years of experimentation can be saved by discovering that a new protein has an amino acid sequence similarity with a protein of known function. Such sequence relationships, for example, first indicated that certain genes that cause mammalian cells to become cancerous are protein kinases. In the same way, many of the proteins that control pattern formation during the embryonic development of the fruit fly *Drosophila* were quickly recognized to be gene regulatory proteins.

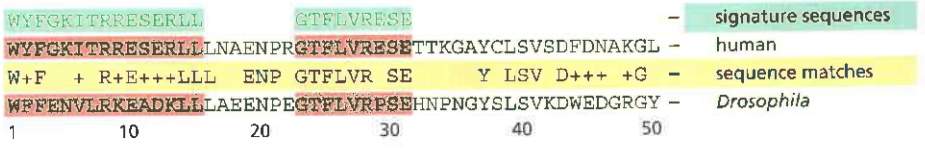


Figure 3–14 The use of short signature sequences to find related protein domains. The two short sequences of 15 and 9 amino acids shown *(green)* can be used to search large databases for a protein domain that is found in many proteins, the SH2 domain. Here, the first 50 amino acids of the SH2 domain of 100 amino acids is compared for the human and *Drosophila* Src protein (see Figure 3–10). In the computer-generated sequence comparison *(yellow row)*, exact matches between the human and *Drosophila* proteins are noted by the one-letter abbreviation for the amino acid; the positions with a similar but nonidentical amino acid are denoted by +, and nonmatches are blank. In this diagram, wherever one or both proteins contain an exact match to a position in the *green* sequences, both aligned sequences are colored *red*.

Some Protein Domains Form Parts of Many Different Proteins

As previously stated, most proteins are composed of a series of protein domains, in which different regions of the polypeptide chain have folded independently to form compact structures. Such multidomain proteins are believed to have originated from the accidental joining of the DNA sequences that encode each domain, creating a new gene. Novel binding surfaces have often been created at the juxtaposition of domains, and many of the functional sites where proteins bind to small molecules are found to be located there. In an evolutionary process called *domain shuffling*, many large proteins have evolved through the joining of preexisting domains in new combinations (**Figure 3–15**).

A subset of protein domains have been especially mobile during evolution; these seem to have particularly versatile structures and are sometimes referred to as *protein modules*. The structure of one such module, the SH2 domain, was illustrated in Panel 3–2 (pp. 132–133). Some other abundant protein domains are illustrated in **Figure 3–16**.

Each of the domains shown has a stable core structure formed from strands of β sheet, from which less-ordered loops of polypeptide chain protrude *(green)*. The loops are ideally situated to form binding sites for other molecules, as most clearly demonstrated for the immunoglobulin fold, which forms the basis for antibody molecules (see Figure 3–41). Most likely, such β-sheet-based domains have achieved their evolutionary success because they provide a convenient framework for the generation of new binding sites for ligands through small changes to their protruding loops.

A second feature of these protein domains that explains their utility is the ease with which they can be integrated into other proteins. Five of the six domains illustrated in Figure 3–16 have their N- and C-terminal ends at opposite poles of the domain. When the DNA encoding such a domain undergoes tandem duplication, which is not unusual in the evolution of genomes (discussed in Chapter 4), the duplicated modules with this "in-line" arrangement

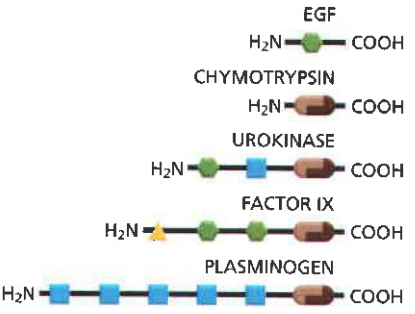


Figure 3–15 Domain shuffling. An extensive shuffling of blocks of protein sequence (protein domains) has occurred during protein evolution. Those portions of a protein denoted by the same shape and color in this diagram are evolutionarily related. Serine proteases like chymotrypsin are formed from two domains *(brown)*. In the three other proteases shown, which are highly regulated and more specialized, these two protease domains are connected to one or more domains that are similar to domains found in epidermal growth factor (EGF; *green*), to a calcium-binding protein *(yellow)*, or to a "kringle" domain *(blue)* that contains three internal disulfide bridges. Chymotrypsin is illustrated in Figure 3–12.

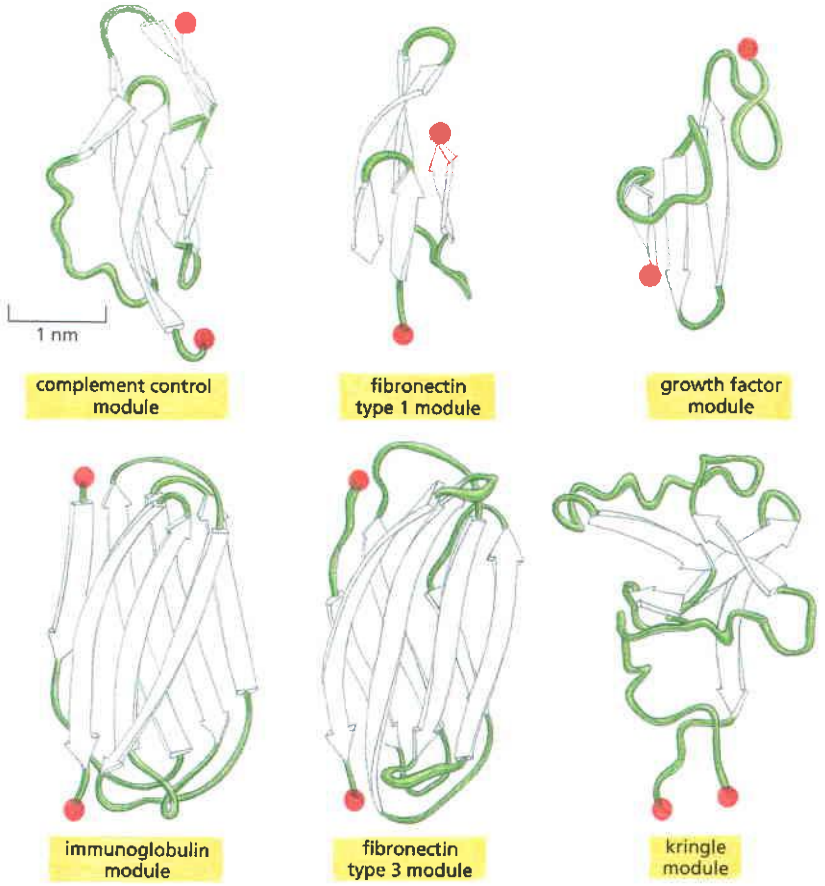


Figure 3–16 The three-dimensional structures of some protein modules. In these ribbon diagrams, β-sheet strands are shown as *arrows*, and the N- and C-termini are indicated by *red spheres*. (Adapted from M. Baron, D.G. Norman and I.D. Campbell, *Trends Biochem. Sci.* 16:13–17, 1991, with permission from Elsevier, and D.J. Leahy et al., *Science* 258:987–991, 1992, with permission from AAAS.)

can be readily linked in series to form extended structures—either with themselves or with other in-line domains (**Figure 3–17**). Stiff extended structures composed of a series of domains are especially common in extracellular matrix molecules and in the extracellular portions of cell-surface receptor proteins. Other modules, including the SH2 domain and the kringle domain illustrated in Figure 3–16, are of a "plug-in" type, with their N- and C-termini close together. After genomic rearrangements, such modules are usually accommodated as an insertion into a loop region of a second protein.

A comparison of the relative frequency of domain utilization in different eucaryotes reveals that, for many common domains, such as protein kinases, this frequency is similar in organisms as diverse as yeast, plants, worms, flies, and humans (**Figure 3–18**). But there are some notable exceptions, such as the Major Histocompatibility Complex (MHC) antigen-recognition domain (see Figure 25–52) that is present in 57 copies in humans, but absent in the other four organisms just mentioned. Such domains presumably have specialized functions that are not shared with the other eucaryotes, being strongly selected for during evolution so as to give rise to the multiple copies observed. Similarly, a domain like SH2 that shows an unusual increase in its numbers in higher eucaryotes might be assumed to be especially useful for multicellularity (compare the multicellular organisms with yeast in Figure 3–18).

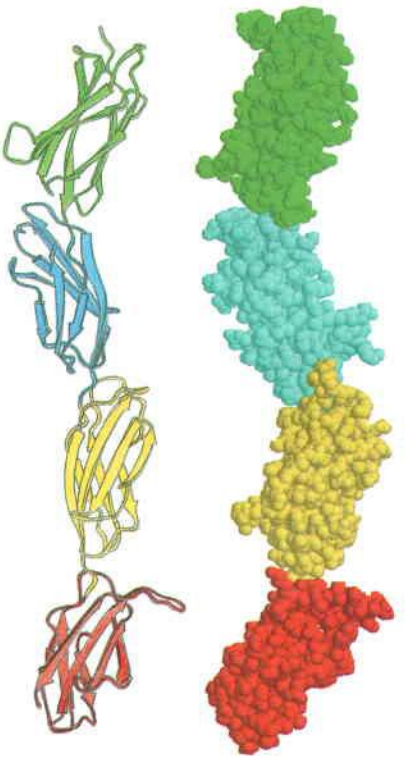


Figure 3–17 An extended structure formed from a series of in-line protein modules. Four fibronectin type 3 modules (see Figure 3–16) from the extracellular matrix molecule fibronectin are illustrated in (A) ribbon and (B) space-filling models. (Adapted from D.J. Leahy, I. Aukhil and H.P. Erickson, *Cell* 84:155–164, 1996. With permission from Elsevier.)

Certain Pairs of Domains Are Found Together in Many Proteins

We can construct a large table displaying domain usage for each organism whose genome sequence is known. For example, the human genome is estimated to contain about 1000 immunoglobulin domains, 500 protein kinase domains, 250 DNA-binding homeodomains, 300 SH3 domains, and 120 SH2 domains. Important additional information can be derived by comparing the frequencies and arrangements of domains in the more than 100 eucaryotic, bacterial, and archaeal genomes that have been completely sequenced. For example, we find that more than two-thirds of proteins consist of two or more domains, and that the same pairs of domains occur repeatedly in the same relative arrangement in a protein. Although half of all domain families are common to archaea, bacteria, and eucaryotes, only about 5 percent of the two-domain combinations are similarly shared. This pattern suggests that most proteins containing especially useful two-domain combinations arose relatively late in evolution.

The 200 most abundant two-domain combinations occur in about one-fourth of all of the proteins with recognizable domains in the complete data set. It would therefore be very useful to determine the precise three-dimensional structure for at least one protein from each common two-domain combination, so as to reveal how the domains interact in that type of protein structure.

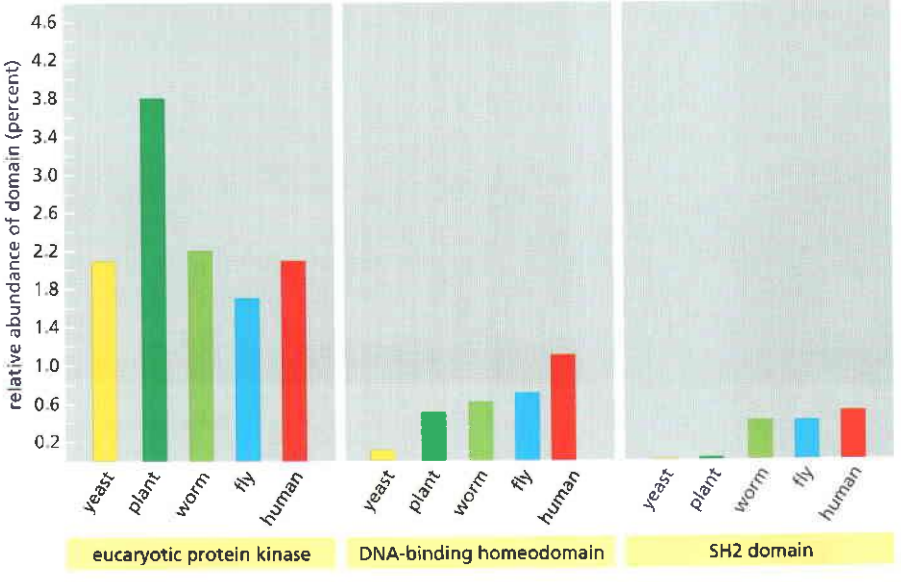


Figure 3–18 The relative frequencies of three protein domains in five eucaryotic organisms. The approximate percentages given have been determined by dividing the number of copies of each domain by the total number of distinct proteins thought to be encoded by each organism, as determined from the sequence of its genome. Thus, for SH2 domains in humans, 120/24,000 = 0.005.

The Human Genome Encodes a Complex Set of Proteins, Revealing Much That Remains Unknown

The result of sequencing the human genome has been surprising, because it reveals that our chromosomes contain only about 25,000 genes. Based on gene number alone, we would appear to be no more complex than the tiny mustard weed, *Arabidopsis*, and only about 1.3-fold more complex than a nematode worm. The genome sequences also reveal that vertebrates have inherited nearly all of their protein domains from invertebrates—with only 7 percent of identified human domains being vertebrate-specific.

Each of our proteins is on average more complicated, however (**Figure 3–19**). Domain shuffling during vertebrate evolution has given rise to many novel combinations of protein domains, with the result that there are nearly twice as many combinations of domains found in human proteins as in a worm or a fly. Thus, for example, the trypsinlike serine protease domain is linked to at least 18 other types of protein domains in human proteins, whereas it is found covalently joined to only 5 different domains in the worm. This extra variety in our proteins greatly increases the range of protein–protein interactions possible (see Figure 3–82), but how it contributes to making us human is not known.

The complexity of living organisms is staggering, and it is quite sobering to note that we currently lack even the tiniest hint of what the function might be for more than 10,000 of the proteins that have thus far been identified in the human genome. There are certainly enormous challenges ahead for the next generation of cell biologists, with no shortage of fascinating mysteries to solve.

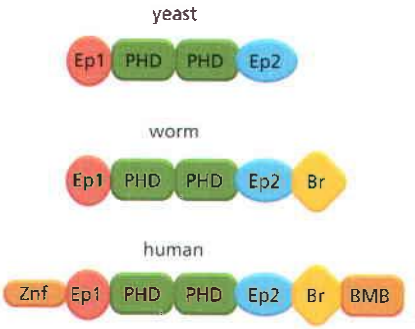


Figure 3–19 Domain structure of a group of evolutionarily related proteins that are thought to have a similar function. In general, there is a tendency for the proteins in more complex organisms, such as humans, to contain additional domains—as is the case for the DNA-binding protein compared here.

Larger Protein Molecules Often Contain More Than One Polypeptide Chain <GCCT>

The same weak noncovalent bonds that enable a protein chain to fold into a specific conformation also allow proteins to bind to each other to produce larger structures in the cell. Any region of a protein's surface that can interact with another molecule through sets of noncovalent bonds is called a **binding site**. A protein can contain binding sites for various large and small molecules. If a binding site recognizes the surface of a second protein, the tight binding of two folded polypeptide chains at this site creates a larger protein molecule with a precisely defined geometry. Each polypeptide chain in such a protein is called a **protein subunit**.

In the simplest case, two identical folded polypeptide chains bind to each other in a "head-to-head" arrangement, forming a symmetric complex of two protein subunits (a *dimer*) held together by interactions between two identical binding sites. The *Cro repressor protein*—a viral gene regulatory protein that binds to DNA to turn viral genes off in an infected bacterial cell—provides an example (**Figure 3–20**). Cells contain many other types of symmetric protein complexes, formed from multiple copies of a single polypeptide chain. The enzyme *neuraminidase*, for example, consists of four identical protein subunits, each bound to the next in a "head-to-tail" arrangement that forms a closed ring (**Figure 3–21**).

Many of the proteins in cells contain two or more types of polypeptide chains. *Hemoglobin*, the protein that carries oxygen in red blood cells, contains

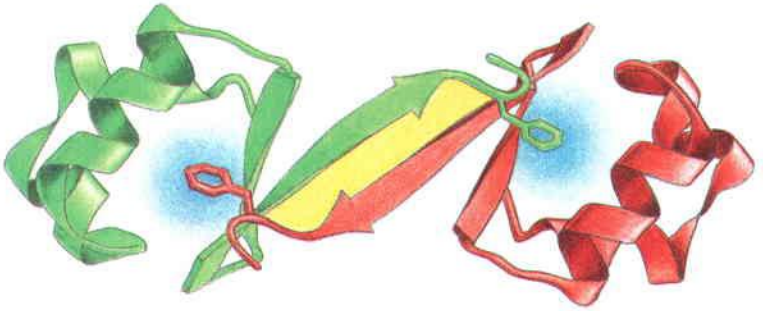


Figure 3–20 Two identical protein subunits binding together to form a symmetric protein dimer. The Cro repressor protein from bacteriophage lambda binds to DNA to turn off viral genes. Its two identical subunits bind head-to-head, held together by a combination of hydrophobic forces *(blue)* and a set of hydrogen bonds *(yellow* region). (Adapted from D.H. Ohlendorf, D.E. Tronrud and B.W. Matthews, *J. Mol. Biol.* 280:129–136, 1998. With permission from Academic Press.)

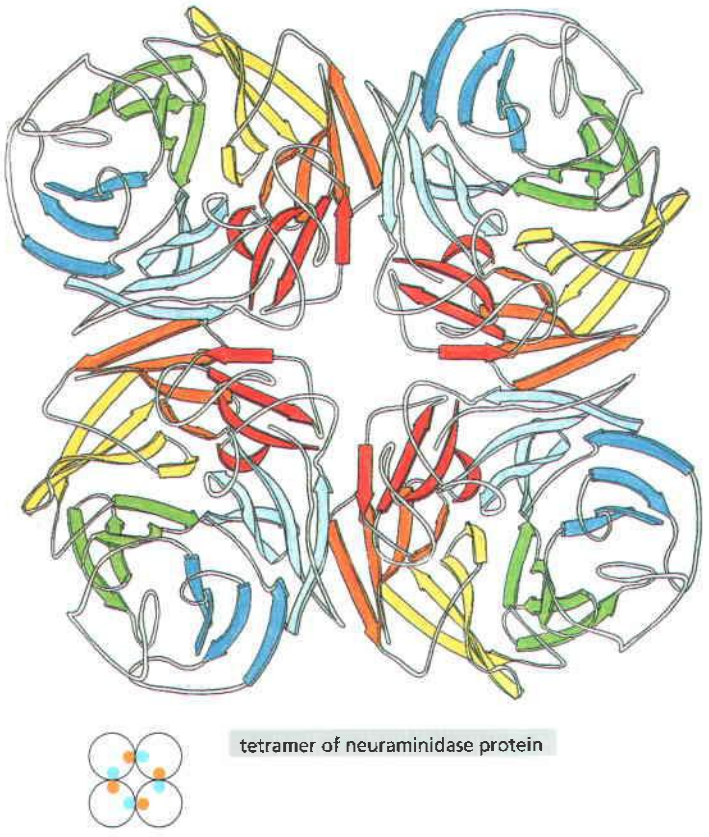


Figure 3–21 A protein molecule containing multiple copies of a single protein subunit. The enzyme neuraminidase exists as a ring of four identical polypeptide chains. Each of these chains is formed from six repeats of a four-stranded β sheet, as indicated by the colored arrows. The small diagram shows how the repeated use of the same binding interaction forms the structure.

two identical α-globin subunits and two identical β-globin subunits, symmetrically arranged (**Figure 3–22**). Such multisubunit proteins are very common in cells, and they can be very large. **Figure 3–23** shows a sample of proteins whose exact structures are known, and it compares the sizes and shapes of a few larger proteins with some of the relatively small proteins that we have thus far presented as models.

Some Proteins Form Long Helical Filaments

Some protein molecules can assemble to form filaments that may span the entire length of a cell. Most simply, a long chain of identical protein molecules can be constructed if each molecule has a binding site complementary to another region of the surface of the same molecule (**Figure 3–24**). An actin filament, for example, is a long helical structure produced from many molecules of the protein *actin* (**Figure 3–25**). Actin is very abundant in eucaryotic cells, where it constitutes one of the major filament systems of the cytoskeleton (discussed in Chapter 16).

Why is a helix such a common structure in biology? As we have seen, biological structures are often formed by linking similar subunits—such as amino acids or protein molecules—into long, repetitive chains. If all the subunits are identical, the neighboring subunits in the chain can often fit together in only one way, adjusting their relative positions to minimize the free energy of the contact between them. As a result, each subunit is positioned in exactly the same way in relation to the next, so that subunit 3 fits onto subunit 2 in the same way that subunit 2 fits onto subunit 1, and so on. Because it is very rare for subunits to join up in a straight line, this arrangement generally results in a helix—a regular structure that resembles a spiral staircase, as illustrated in **Figure 3–26**. Depending on the twist of the staircase, a helix is said to be either right-handed or left-handed (see Figure 3–26E). Handedness is not affected by turning the helix upside down, but it is reversed if the helix is reflected in the mirror.

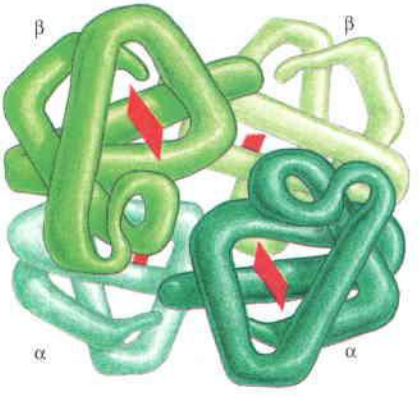


Figure 3–22 A protein formed as a symmetric assembly of two different subunits. Hemoglobin is an abundant protein in red blood cells that contains two copies of α-globin and two copies of β-globin. Each of these four polypeptide chains contains a heme molecule *(red)*, which is the site that binds oxygen (O_2). Thus, each molecule of hemoglobin in the blood carries four molecules of oxygen.

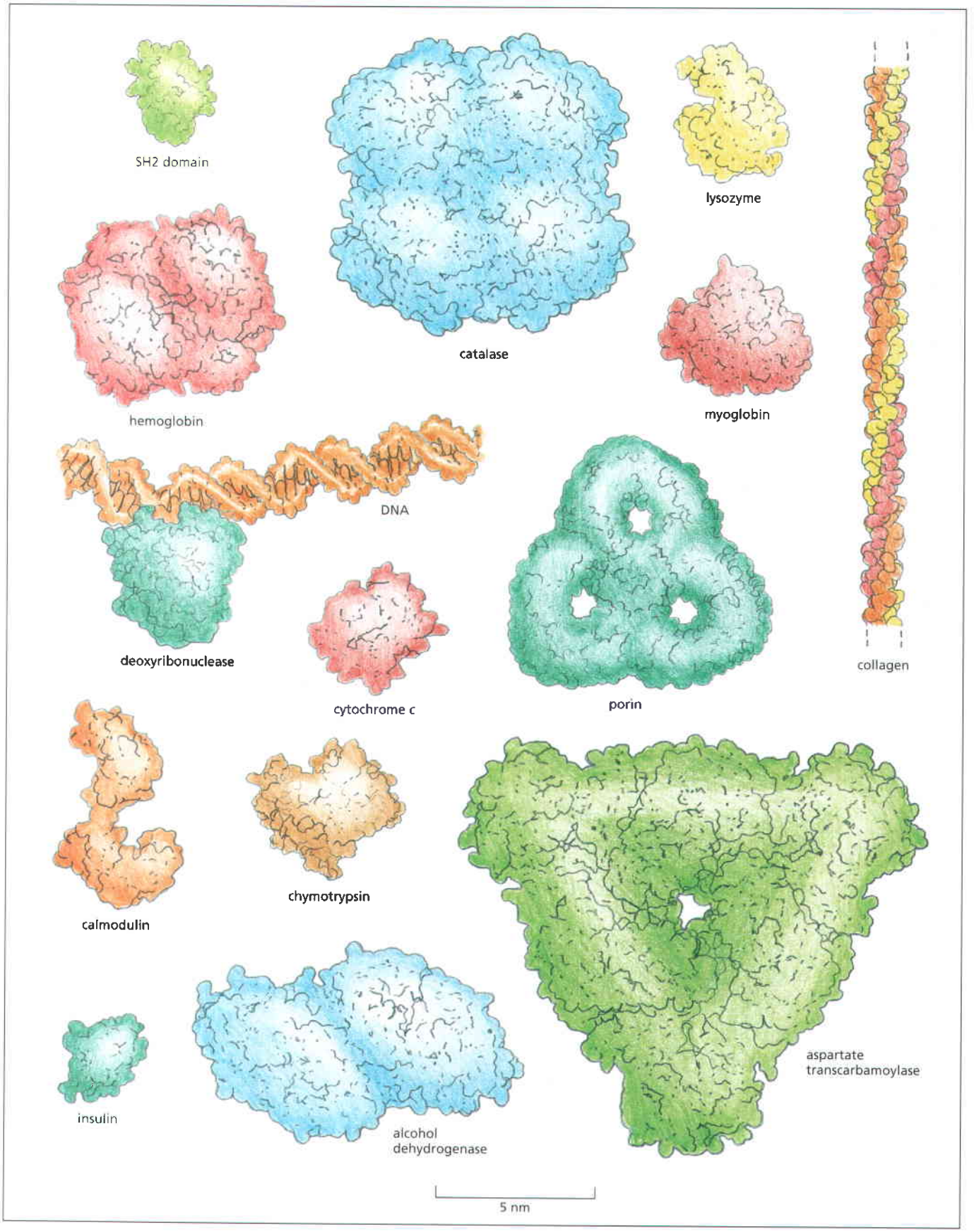


Figure 3–23 A collection of protein molecules, shown at the same scale. For comparison, a DNA molecule bound to a protein is also illustrated. These space-filling models represent a range of sizes and shapes. Hemoglobin, catalase, porin, alcohol dehydrogenase, and aspartate transcarbamoylase are formed from multiple copies of subunits. The SH2 domain *(top left)* is presented in detail in Panel 3–2 (pp. 132–133). (Adapted from David S. Goodsell, Our Molecular Nature. New York: Springer-Verlag, 1996. With permission from Springer Science and Business Media.)

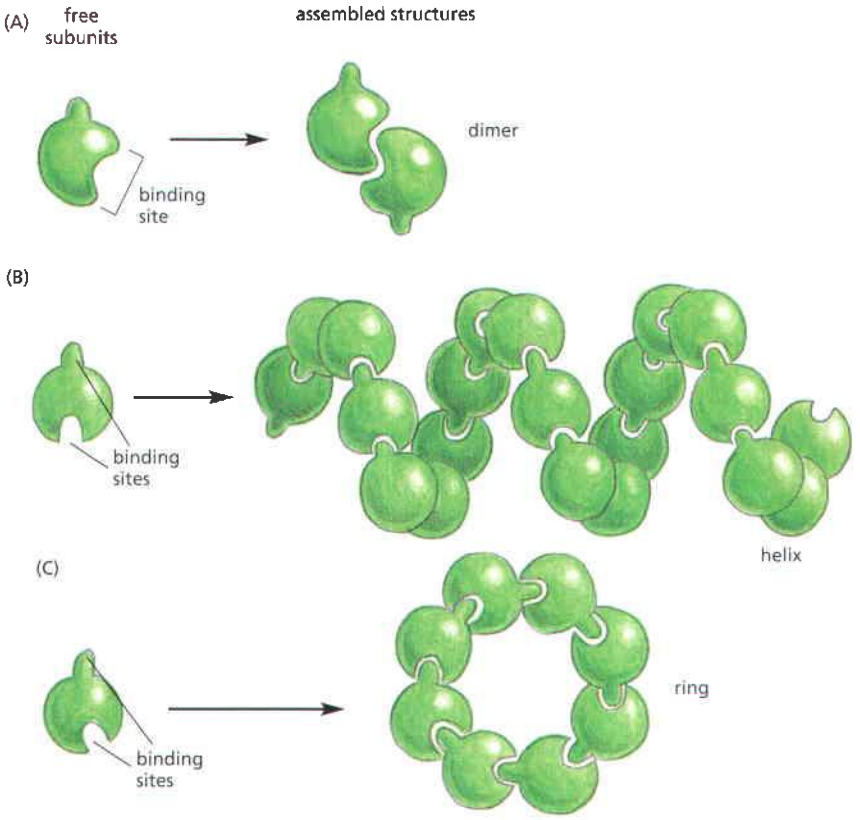


Figure 3–24 Protein assemblies. (A) A protein with just one binding site can form a dimer with another identical protein. (B) Identical proteins with two different binding sites often form a long helical filament. (C) If the two binding sites are disposed appropriately in relation to each other, the protein subunits may form a closed ring instead of a helix. (For an example of A, see Figure 3–20; for an example of C, see Figure 3–21.)

Helices occur commonly in biological structures, whether the subunits are small molecules linked together by covalent bonds (for example, the amino acids in an α helix) or large protein molecules that are linked by noncovalent forces (for example, the actin molecules in actin filaments). This is not surprising. A helix is an unexceptional structure, and it is generated simply by placing many similar subunits next to each other, each in the same strictly repeated relationship to the one before—that is, with a fixed rotation followed by a fixed translation along the helix axis, as in a spiral staircase.

Many Protein Molecules Have Elongated, Fibrous Shapes

Most of the proteins that we have discussed so far are *globular proteins*, in which the polypeptide chain folds up into a compact shape like a ball with an irregular surface. Enzymes tend to be globular proteins: even though many are large and complicated, with multiple subunits, most have an overall rounded shape (see Figure 3–23). In contrast, other proteins have roles in the cell that require each individual protein molecule to span a large distance. These proteins generally have a relatively simple, elongated three-dimensional structure and are commonly referred to as *fibrous proteins*.

One large family of intracellular fibrous proteins consists of α-keratin, introduced when we presented the α helix, and its relatives. Keratin filaments are extremely stable and are the main component in long-lived structures such as hair, horn, and nails. An α-keratin molecule is a dimer of two identical subunits, with the long α helices of each subunit forming a coiled-coil (see Figure 3–9). The coiled-coil regions are capped at each end by globular domains containing binding sites. This enables this class of protein to assemble into ropelike *intermediate filaments*—an important component of the cytoskeleton that creates the cell's internal structural framework (see Figure 16–19).

Fibrous proteins are especially abundant outside the cell, where they are a main component of the gel-like *extracellular matrix* that helps to bind collections of cells together to form tissues. Cells secrete extracellular matrix proteins into their surroundings, where they often assemble into sheets or long fibrils.

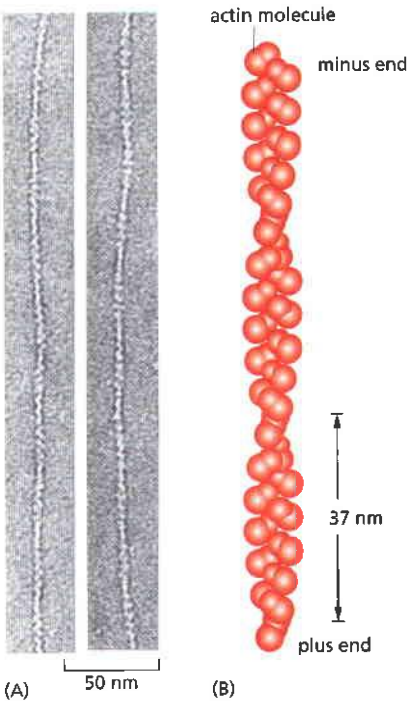


Figure 3–25 Actin filaments. (A) Transmission electron micrographs of negatively stained actin filaments. (B) The helical arrangement of actin molecules in an actin filament. (A, courtesy of Roger Craig.)

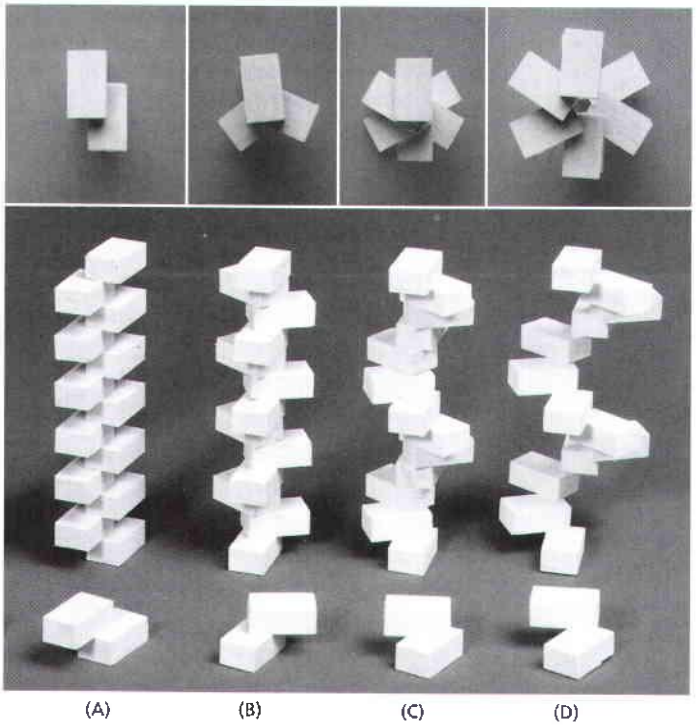
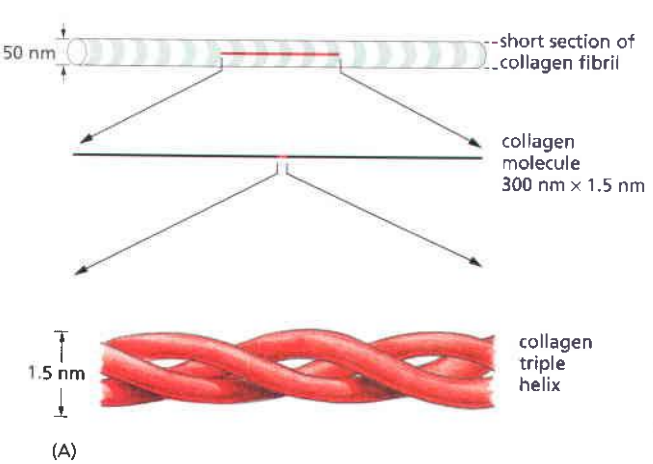


Figure 3–26 Some properties of a helix. (A–D) A helix forms when a series of subunits bind to each other in a regular way. At the bottom, the interaction between two subunits is shown; behind them are the helices that result. These helices have two (A), three (B), and six (C and D) subunits per helical turn. The photographs at the top show the arrangement of subunits viewed from directly above the helix. Note that the helix in (D) has a wider path than that in (C), but the same number of subunits per turn. (E) A helix can be either right-handed or left-handed. As a reference, it is useful to remember that standard metal screws, which insert when turned clockwise, are right-handed. Note that a helix retains the same handedness when it is turned upside down.

Collagen is the most abundant of these proteins in animal tissues. A collagen molecule consists of three long polypeptide chains, each containing the non-polar amino acid glycine at every third position. This regular structure allows the chains to wind around one another to generate a long regular triple helix (**Figure 3–27**A). Many collagen molecules then bind to one another side-by-side and end-to-end to create long overlapping arrays—thereby generating the extremely tough collagen fibrils that give connective tissues their tensile strength, as described in Chapter 19.

Many Proteins Contain a Surprisingly Large Amount of Unstructured Polypeptide Chain

It has been well known for a long time that, in complete contrast to collagen, another abundant protein in the extracellular matrix, *elastin*, is formed as a highly disordered polypeptide. This disorder is essential for elastin's function. Its



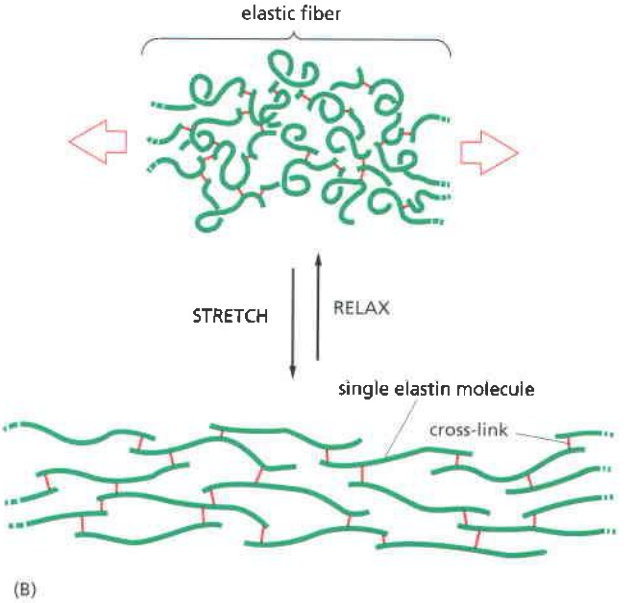


Figure 3–27 Collagen and elastin. (A) Collagen is a triple helix formed by three extended protein chains that wrap around one another *(bottom)*. Many rodlike collagen molecules are cross-linked together in the extracellular space to form unextendable collagen fibrils *(top)* that have the tensile strength of steel. The striping on the collagen fibril is caused by the regular repeating arrangement of the collagen molecules within the fibril. (B) Elastin polypeptide chains are cross-linked together to form rubberlike, elastic fibers. Each elastin molecule uncoils into a more extended conformation when the fiber is stretched and recoils spontaneously as soon as the stretching force is relaxed.

relatively loose and unstructured polypeptide chains are covalently cross-linked to produce a rubberlike elastic meshwork that can be reversibly pulled from one conformation to another, as illustrated in Figure 3–27B. The elastic fibers that result enable skin and other tissues, such as arteries and lungs, to stretch and recoil without tearing.

Intrinsically unstructured regions of proteins are quite frequent in nature, having important functions in the interior of cells. As we have already seen, proteins use the short loops of polypeptide chain that generally protrude from the core region of protein domains to bind other molecules. Similarly, many proteins have much longer regions of unstructured amino acid sequences that interact with another molecule (often DNA or a protein), undergoing a structural transition to a specific folded conformation when the other molecule is bound. Other proteins appear to resemble elastin, in so far as their function requires that they remain largely unstructured. For example, the abundant nucleoporins that coat the inner surface of the nuclear pore complex form a random coil meshwork that is intimately involved in nuclear transport (see Figure 12–10). Finally, as will be discussed later in this chapter (see Figure 3–80C), unstructured regions of polypeptide chain are often used to connect the binding sites for proteins that function together to catalyze a biological reaction. Thus, for example, in facilitating cell signaling, large *scaffold proteins* use such flexible regions as "tethers" that concentrate sets of interacting proteins, often confining them to particular sites in the cell (discussed in Chapter 15).

We can recognize the unstructured regions in many proteins by their biased amino acid composition: they contain very few of the bulky hydrophobic amino acids that normally form the core of a folded protein, being composed instead of a high proportion of the amino acids Gln, Ser, Pro, Glu, and Lys. Such "natively unfolded" regions also frequently contain repeated sequences of amino acids.

Covalent Cross-Linkages Often Stabilize Extracellular Proteins

Many protein molecules are either attached to the outside of a cell's plasma membrane or secreted as part of the extracellular matrix. All such proteins are directly exposed to extracellular conditions. To help maintain their structures, the polypeptide chains in such proteins are often stabilized by covalent cross-linkages. These linkages can either tie two amino acids in the same protein together, or connect different polypeptide chains in a multisubunit protein. The most common cross-linkages in proteins are covalent sulfur–sulfur bonds. These *disulfide bonds* (also called *S–S bonds*) form as cells prepare newly synthesized proteins for export. As described in Chapter 12, their formation is catalyzed in the endoplasmic reticulum by an enzyme that links together two pairs of –SH groups of cysteine side chains that are adjacent in the folded protein (**Figure 3–28**). Disulfide bonds do not change the conformation of a protein but instead act as atomic staples to reinforce its most favored conformation. For

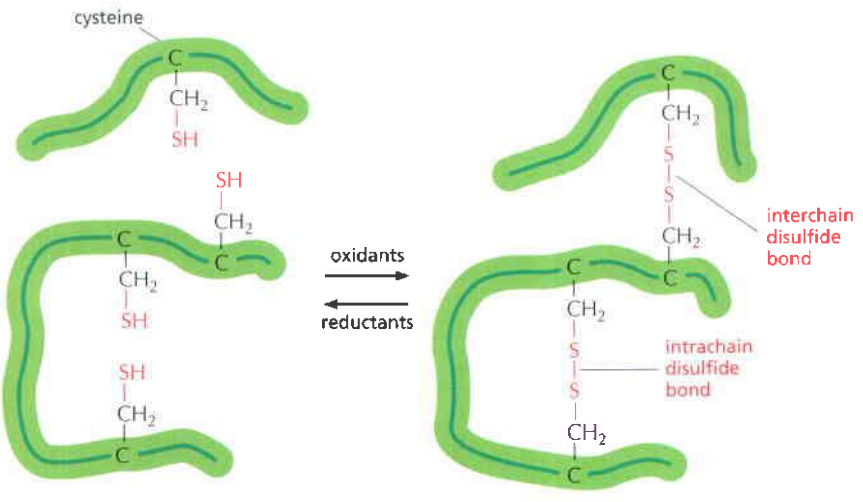


Figure 3–28 Disulfide bonds. <ATAC> This diagram illustrates how covalent disulfide bonds form between adjacent cysteine side chains. As indicated, these cross-linkages can join either two parts of the same polypeptide chain or two different polypeptide chains. Since the energy required to break one covalent bond is much larger than the energy required to break even a whole set of noncovalent bonds (see Table 2–1, p. 53), a disulfide bond can have a major stabilizing effect on a protein.

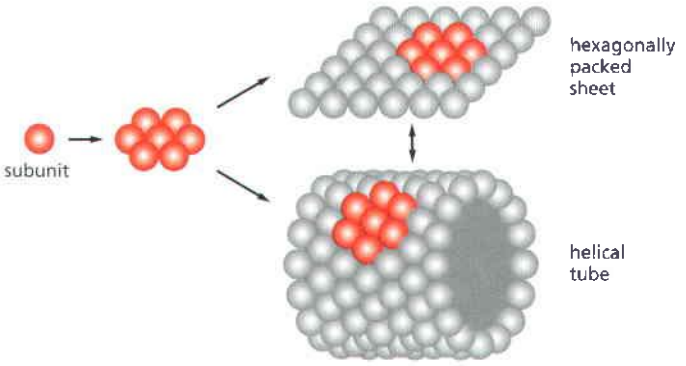


Figure 3–29 An example of the assembly of a single protein subunit requiring multiple protein–protein contacts. Hexagonally packed globular protein subunits can form either a flat sheet or a tube.

example, lysozyme—an enzyme in tears that dissolves bacterial cell walls—retains its antibacterial activity for a long time because it is stabilized by such cross-linkages.

Disulfide bonds generally fail to form in the cell cytosol, where a high concentration of reducing agents converts S–S bonds back to cysteine –SH groups. Apparently, proteins do not require this type of reinforcement in the relatively mild environment inside the cell.

Protein Molecules Often Serve as Subunits for the Assembly of Large Structures

The same principles that enable a protein molecule to associate with itself to form rings or filaments also operate to generate much larger structures in the cell—supramolecular structures such as enzyme complexes, ribosomes, protein filaments, viruses, and membranes. These large objects are not made as single, giant, covalently linked molecules. Instead they are formed by the noncovalent assembly of many separately manufactured molecules, which serve as the subunits of the final structure.

The use of smaller subunits to build larger structures has several advantages:

1. A large structure built from one or a few repeating smaller subunits requires only a small amount of genetic information.
2. Both assembly and disassembly can be readily controlled, reversible processes, because the subunits associate through multiple bonds of relatively low energy.
3. Errors in the synthesis of the structure can be more easily avoided, since correction mechanisms can operate during the course of assembly to exclude malformed subunits.

Some protein subunits assemble into flat sheets in which the subunits are arranged in hexagonal patterns. Specialized membrane proteins are sometimes arranged this way in lipid bilayers. With a slight change in the geometry of the individual subunits, a hexagonal sheet can be converted into a tube (**Figure 3–29**) or, with more changes, into a hollow sphere. Protein tubes and spheres that bind specific RNA and DNA molecules in their interior form the coats of viruses.

The formation of closed structures, such as rings, tubes, or spheres, provides additional stability because it increases the number of bonds between the protein subunits. Moreover, because such a structure is created by mutually dependent, cooperative interactions between subunits, a relatively small change that affects each subunit individually can cause the structure to assemble or disassemble. These principles are dramatically illustrated in the protein coat or *capsid* of many simple viruses, which takes the form of a hollow sphere based on an icosahedron (**Figure 3–30**). Capsids are often made of hundreds of identical protein subunits that enclose and protect the viral nucleic acid (**Figure 3–31**). The protein in such a capsid must have a particularly adaptable structure: not only must it make several different kinds of contacts to create the sphere, it must also change this arrangement to let the nucleic acid out to initiate viral replication once the virus has entered a cell.

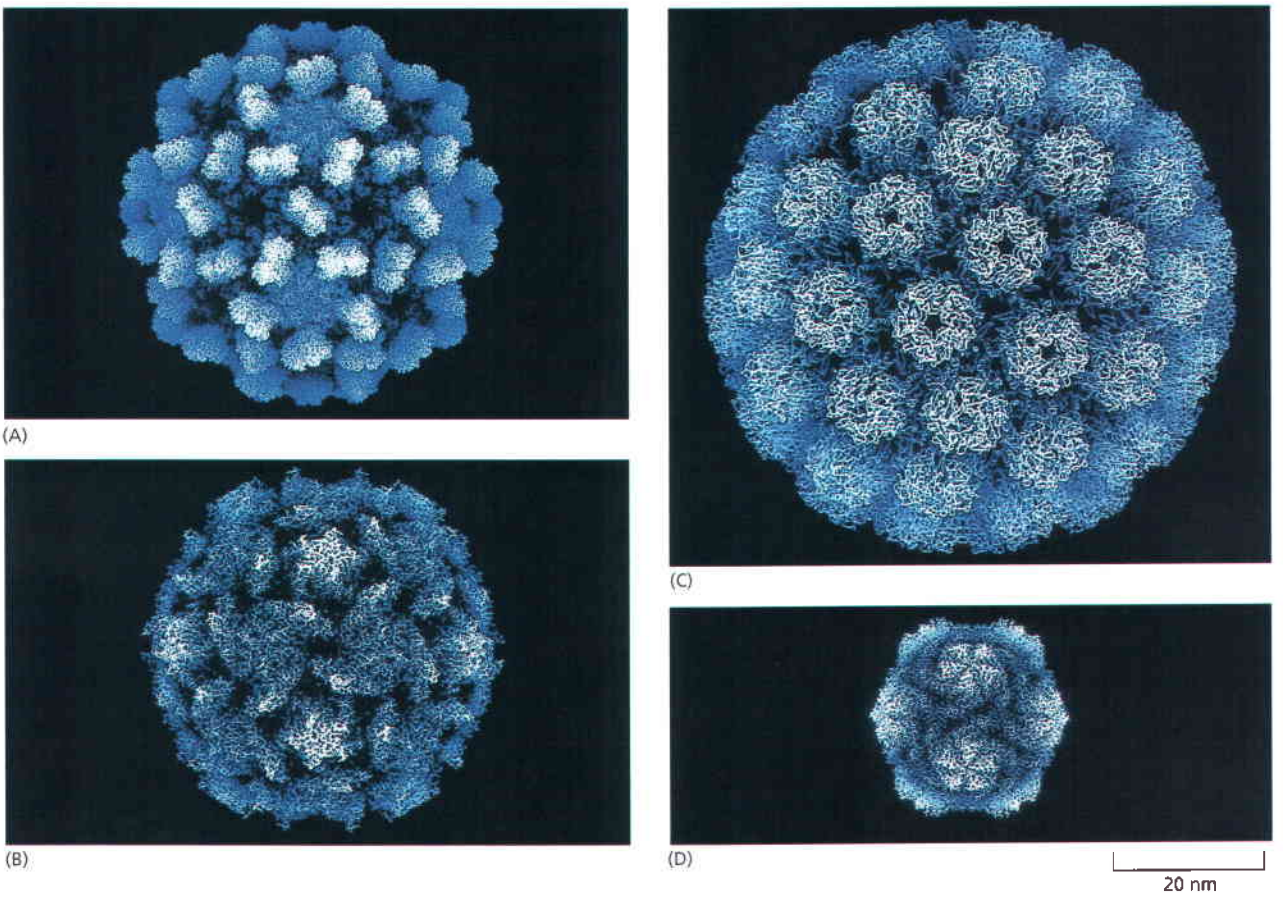


Figure 3–30 The capsids of some viruses, all shown at the same scale. (A) Tomato bushy stunt virus; (B) poliovirus; (C) simian virus 40 (SV40); (D) satellite tobacco necrosis virus. The structures of all of these capsids have been determined by x-ray crystallography and are known in atomic detail. (Courtesy of Robert Grant, Stephan Crainic, and James M. Hogle.)

Many Structures in Cells Are Capable of Self-Assembly

The information for forming many of the complex assemblies of macromolecules in cells must be contained in the subunits themselves, because purified subunits can spontaneously assemble into the final structure under the appropriate conditions. The first large macromolecular aggregate shown to be capable of self-assembly from its component parts was *tobacco mosaic virus (TMV)*. This virus is a long rod in which a cylinder of protein is arranged around a helical RNA core (**Figure 3–32**). If the dissociated RNA and protein subunits are mixed together in solution, they recombine to form fully active viral particles. The assembly process is unexpectedly complex and includes the formation of double rings of protein, which serve as intermediates that add to the growing viral coat.

Another complex macromolecular aggregate that can reassemble from its component parts is the bacterial ribosome. This structure is composed of about 55 different protein molecules and 3 different rRNA molecules. Incubating the individual components under appropriate conditions in a test tube causes them to spontaneously re-form the original structure. Most importantly, such reconstituted ribosomes are able to catalyze protein synthesis. As might be expected, the reassembly of ribosomes follows a specific pathway: after certain proteins have bound to the RNA, this complex is then recognized by other proteins, and so on, until the structure is complete.

It is still not clear how some of the more elaborate self-assembly processes are regulated. Many structures in the cell, for example, seem to have a precisely

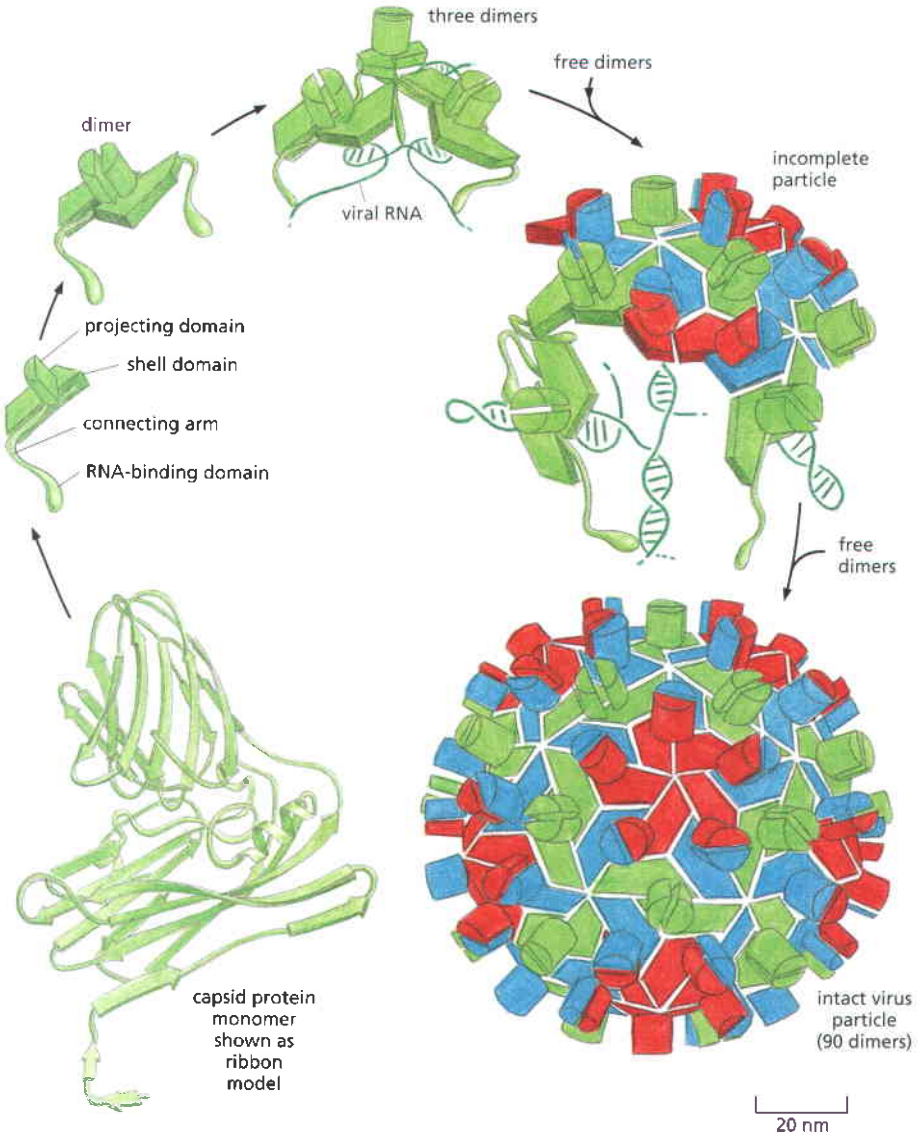
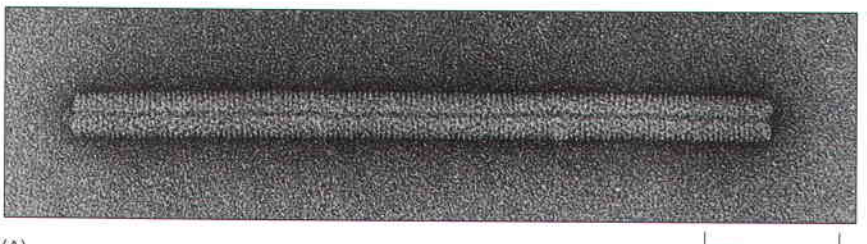


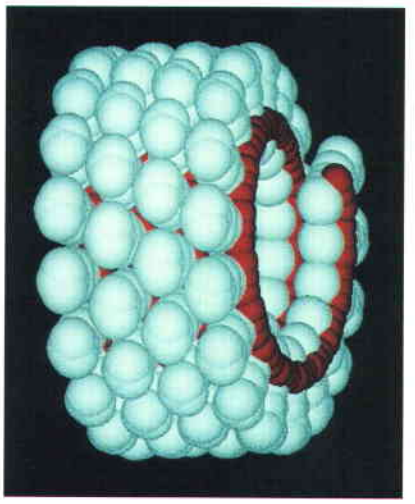
Figure 3–31 The structure of a spherical virus. In many viruses, identical protein subunits pack together to create a spherical shell (a capsid) that encloses the viral genome, composed of either RNA or DNA (see also Figure 3–30). For geometric reasons, no more than 60 identical subunits can pack together in a precisely symmetric way. If slight irregularities are allowed, however, more subunits can be used to produce a larger capsid that retains icosahedral symmetry. The tomato bushy stunt virus (TBSV) shown here, for example, is a spherical virus about 33 nm in diameter formed from 180 identical copies of a 386 amino acid capsid protein plus an RNA genome of 4500 nucleotides. To construct such a large capsid, the protein must be able to fit into three somewhat different environments, each of which is differently colored in the virus particle shown here. The postulated pathway of assembly is shown; the precise three-dimensional structure has been determined by x-ray diffraction. (Courtesy of Steve Harrison.)

defined length that is many times greater than that of their component macromolecules. How such length determination is achieved is in many cases a mystery. Three possible mechanisms are illustrated in Figure 3–33. In the simplest

Figure 3–32 The structure of tobacco mosaic virus (TMV). (A) An electron micrograph of the viral particle, which consists of a single long RNA molecule enclosed in a cylindrical protein coat composed of identical protein subunits. (B) A model showing part of the structure of TMV. A single-stranded RNA molecule of 6395 nucleotides is packaged in a helical coat constructed from 2130 copies of a coat protein 158 amino acids long. Fully infective viral particles can self-assemble in a test tube from purified RNA and protein molecules. (A, courtesy of Robley Williams; B, courtesy of Richard J. Feldmann.)



(A)



(B)

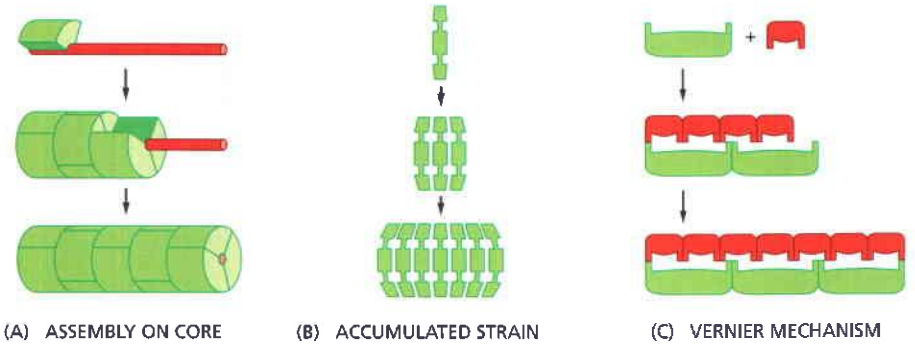


Figure 3–33 Three mechanisms of length determination for large protein assemblies. (A) Coassembly along an elongated core protein or other macromolecule that acts as a measuring device. (B) Termination of assembly because of strain that accumulates in the polymeric structure as additional subunits are added, so that beyond a certain length the energy required to fit another subunit onto the chain becomes excessively large. (C) A vernier type of assembly, in which two sets of rodlike molecules differing in length form a staggered complex that grows until their ends exactly match. The name derives from a measuring device based on the same principle, used in mechanical instruments.

case, a long core protein or other macromolecule provides a scaffold that determines the extent of the final assembly. This is the mechanism that determines the length of the TMV particle, where the RNA chain provides the core. Similarly, a core protein is thought to determine the length of the thin filaments in muscle, as well as the length of the long tails of some bacterial viruses (**Figure 3–34**).

Assembly Factors Often Aid the Formation of Complex Biological Structures

Not all cellular structures held together by noncovalent bonds self-assemble. A mitochondrion, a cilium, or a myofibril of a muscle cell, for example, cannot form spontaneously from a solution of its component macromolecules. In these cases, part of the assembly information is provided by special enzymes and other proteins that perform the function of templates, guiding construction but taking no part in the final assembled structure.

Even relatively simple structures may lack some of the ingredients necessary for their own assembly. In the formation of certain bacterial viruses, for example, the head, which is composed of many copies of a single protein subunit, is assembled on a temporary scaffold composed of a second protein. Because the second protein is absent from the final viral particle, the head structure cannot spontaneously reassemble once it has been taken apart. Other examples are known in which proteolytic cleavage is an essential and irreversible step in the normal assembly process. This is even the case for some small protein assemblies, including the structural protein collagen and the hormone insulin (**Figure 3–35**). From these relatively simple examples, it seems certain that the assembly of a structure as complex as a mitochondrion or a cilium will involve temporal and spatial ordering imparted by numerous other cell components.

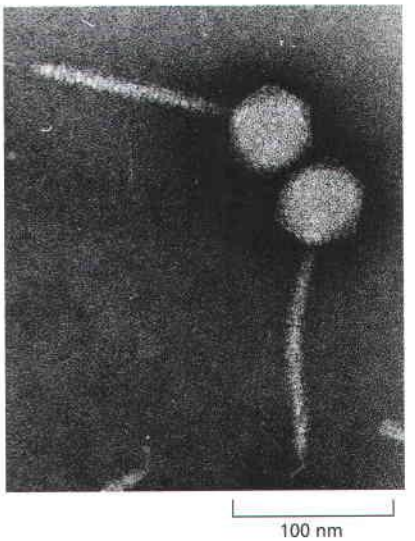


Figure 3–34 An electron micrograph of bacteriophage lambda. The tip of the virus tail attaches to a specific protein on the surface of a bacterial cell, after which the tightly packaged DNA in the head is injected through the tail into the cell. The tail has a precise length, determined by the mechanism shown in Figure 3–33A.

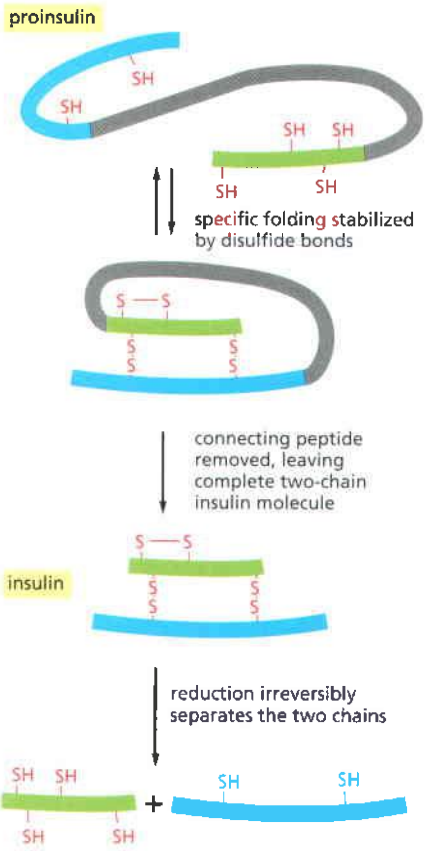


Figure 3–35 Proteolytic cleavage in insulin assembly. The polypeptide hormone insulin cannot spontaneously re-form efficiently if its disulfide bonds are disrupted. It is synthesized as a larger protein *(proinsulin)* that is cleaved by a proteolytic enzyme after the protein chain has folded into a specific shape. Excision of part of the proinsulin polypeptide chain removes some of the information needed for the protein to fold spontaneously into its normal conformation. Once insulin has been denatured and its two polypeptide chains have separated, its ability to reassemble is lost.

Summary

A protein molecule's amino acid sequence determines its three-dimensional conformation. Noncovalent interactions between different parts of the polypeptide chain stabilize its folded structure. The amino acids with hydrophobic side chains tend to cluster in the interior of the molecule, and local hydrogen-bond interactions between neighboring peptide bonds give rise to α helices and β sheets.

Globular regions, known as domains, are the modular units from which many proteins are constructed; such domains generally contain 40–350 amino acids. Small proteins typically consist of only a single domain, while large proteins are formed from several domains linked together by various lengths of polypeptide chain, some of which can be relatively disordered. As proteins have evolved, domains have been modified and combined with other domains to construct new proteins. Thus far, about 800 different ways of folding up a domain have been observed, among more than 20,000 known protein structures.

Proteins are brought together into larger structures by the same noncovalent forces that determine protein folding. Proteins with binding sites for their own surface can assemble into dimers, closed rings, spherical shells, or helical polymers. Although mixtures of proteins and nucleic acids can assemble spontaneously into complex structures in a test tube, many biological assembly processes involve irreversible steps. Consequently, not all structures in the cell are capable of spontaneous reassembly after they have been dissociated into their component parts.

PROTEIN FUNCTION

We have seen that each type of protein consists of a precise sequence of amino acids that allows it to fold up into a particular three-dimensional shape, or conformation. But proteins are not rigid lumps of material. They often have precisely engineered moving parts whose mechanical actions are coupled to chemical events. It is this coupling of chemistry and movement that gives proteins the extraordinary capabilities that underlie the dynamic processes in living cells.

In this section, we explain how proteins bind to other selected molecules and how their activity depends on such binding. We show that the ability to bind to other molecules enables proteins to act as catalysts, signal receptors, switches, motors, or tiny pumps. The examples we discuss in this chapter by no means exhaust the vast functional repertoire of proteins. You will encounter the specialized functions of many other proteins elsewhere in this book, based on similar principles.

All Proteins Bind to Other Molecules

A protein molecule's physical interaction with other molecules determines its biological properties. Thus, antibodies attach to viruses or bacteria to mark them for destruction, the enzyme hexokinase binds glucose and ATP so as to catalyze a reaction between them, actin molecules bind to each other to assemble into actin filaments, and so on. Indeed, all proteins stick, or *bind*, to other molecules. In some cases, this binding is very tight; in others it is weak and short-lived. But the binding always shows great *specificity*, in the sense that each protein molecule can usually bind just one or a few molecules out of the many thousands of different types it encounters. The substance that is bound by the protein—whether it is an ion, a small molecule, or a macromolecule such as another protein—is referred to as a **ligand** for that protein (from the Latin word *ligare*, meaning "to bind").

The ability of a protein to bind selectively and with high affinity to a ligand depends on the formation of a set of weak, noncovalent bonds—hydrogen bonds, electrostatic attractions, and van der Waals attractions—plus favorable hydrophobic interactions (see Panel 2–3, pp. 110–111). Because each individual bond is weak, effective binding occurs only when many of these bonds form simultaneously. Such binding is possible only if the surface contours of the ligand molecule fit very closely to the protein, matching it like a hand in a glove (**Figure 3–36**).

The region of a protein that associates with a ligand, known as the ligand's *binding site*, usually consists of a cavity in the protein surface formed by a particular arrangement of amino acids. These amino acids can belong to different portions of the polypeptide chain that are brought together when the protein folds (**Figure 3–37**). Separate regions of the protein surface generally provide binding sites for different ligands, allowing the protein's activity to be regulated, as we shall see later. And other parts of the protein act as a handle to position the protein in the cell—an example is the SH2 domain discussed previously, which often moves a protein containing it to particular intracellular sites in response to particular signals.

Although the atoms buried in the interior of the protein have no direct contact with the ligand, they form the framework that gives the surface its contours and its chemical and mechanical properties. Even small changes to the amino acids in the interior of a protein molecule can change its three-dimensional shape enough to destroy a binding site on the surface.

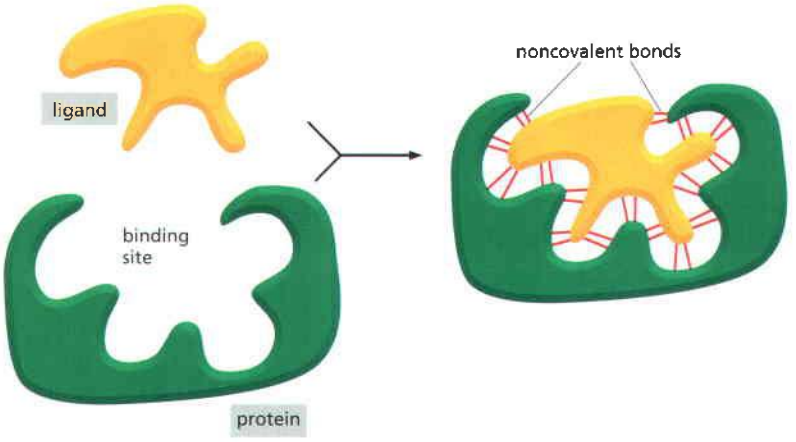


Figure 3–36 The selective binding of a protein to another molecule. Many weak bonds are needed to enable a protein to bind tightly to a second molecule, which is called a *ligand* for the protein. A ligand must therefore fit precisely into a protein's binding site, like a hand into a glove, so that a large number of noncovalent bonds form between the protein and the ligand.

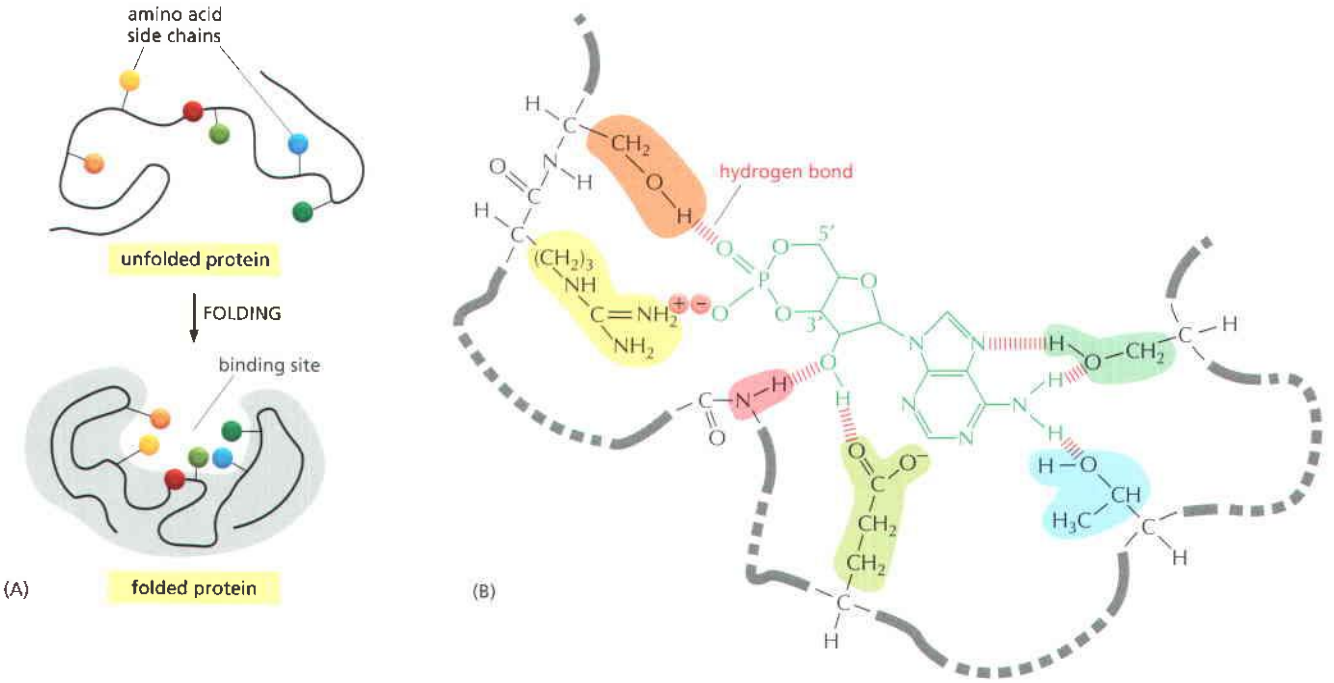


Figure 3–37 The binding site of a protein. (A) The folding of the polypeptide chain typically creates a crevice or cavity on the protein surface. This crevice contains a set of amino acid side chains disposed in such a way that they can form noncovalent bonds only with certain ligands. (B) A close-up of an actual binding site showing the hydrogen bonds and electrostatic interactions formed between a protein and its ligand. In this example, cyclic AMP is the bound ligand.

The Surface Conformation of a Protein Determines Its Chemistry

Proteins have impressive chemical capabilities because the neighboring chemical groups on their surface often interact in ways that enhance the chemical reactivity of amino acid side chains. These interactions fall into two main categories.

First, the interaction of neighboring parts of the polypeptide chain may restrict the access of water molecules to that protein's ligand-binding sites. This is important because water molecules readily form hydrogen bonds that can compete with ligands for sites on the protein surface. Proteins and their ligands form tighter hydrogen bonds (and electrostatic interactions) if the protein can exclude water molecules from its binding sites. It might be hard to imagine a mechanism that would exclude a molecule as small as water from a protein surface without affecting the access of the ligand itself. However, because of the strong tendency of water molecules to form water–water hydrogen bonds, water molecules exist in a large hydrogen-bonded network (see Panel 2–2, pp. 108–109). In effect, a protein can keep a ligand-binding site dry because it is energetically unfavorable for individual water molecules to break away from this network, as they must do to reach into a crevice on a protein's surface.

Second, the clustering of neighboring polar amino acid side chains can alter their reactivity. If protein folding forces together a number of negatively charged side chains against their mutual repulsion, for example, the affinity of the site for a positively charged ion is greatly increased. In addition, when amino acid side chains interact with one another through hydrogen bonds, normally unreactive side groups (such as the $-CH_2OH$ on the serine shown in **Figure 3–38**) can become reactive, enabling them to be used to make or break selected covalent bonds.

The surface of each protein molecule therefore has a unique chemical reactivity that depends not only on which amino acid side chains are exposed, but

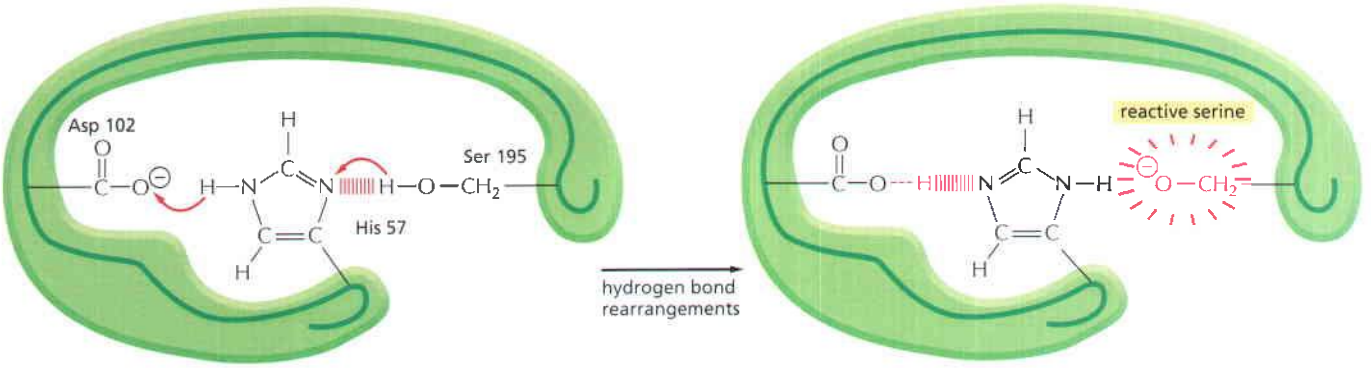


Figure 3–38 An unusually reactive amino acid at the active site of an enzyme. This example is the "catalytic triad" found in chymotrypsin, elastase, and other serine proteases (see Figure 3–12). The aspartic acid side chain (Asp 102) induces the histidine (His 57) to remove the proton from serine 195. This activates the serine to form a covalent bond with the enzyme substrate, hydrolyzing a peptide bond. The many convolutions of the polypeptide chain are omitted here.

also on their exact orientation relative to one another. For this reason, two even slightly different conformations of the same protein molecule may differ greatly in their chemistry.

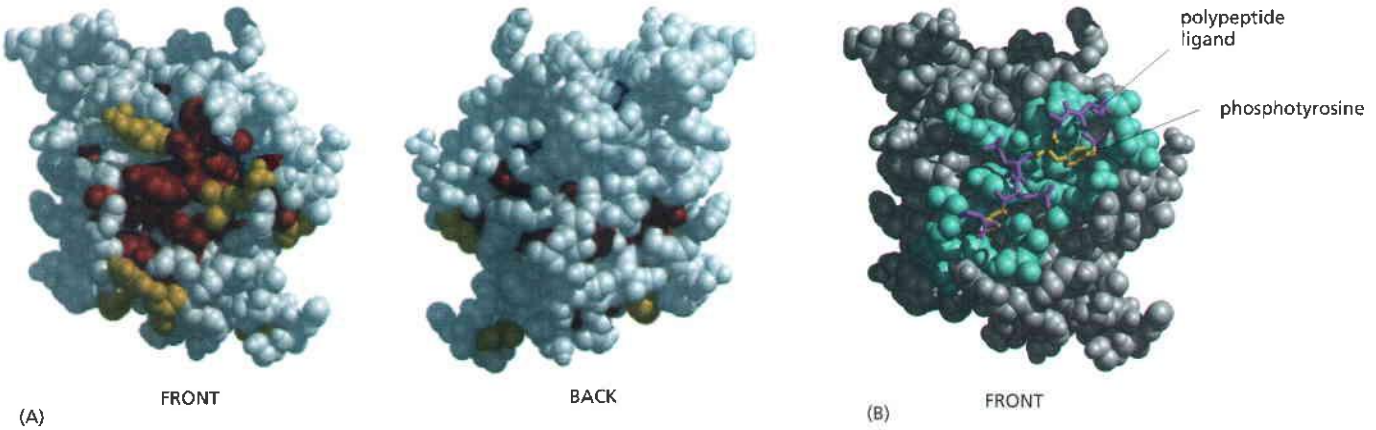
Sequence Comparisons Between Protein Family Members Highlight Crucial Ligand-Binding Sites

As we have described previously, genome sequences allow us to group many of the domains in proteins into families that show clear evidence of their evolution from a common ancestor. The three-dimensional structures of the members of the same domain family are remarkably similar. For example, even when the amino acid sequence identity falls to 25%, the backbone atoms in a domain follow a common protein fold within 0.2 nanometers (2 Å).

We can therefore use a method called evolutionary tracing to identify those sites in a protein domain that are the most crucial to the domain's function. For this purpose, those amino acids that are unchanged, or nearly unchanged, in all of the known protein family members are mapped onto a model of the three-dimensional structure of one family member. When this is done, the most invariant positions often form one or more clusters on the protein surface, as illustrated in **Figure 3–39A** for the SH2 domain described previously (see Panel 3–2, pp. 132–133). These clusters generally correspond to ligand binding sites.

The SH2 domain is a module that functions in protein–protein interactions. It binds the protein containing it to a second protein that contains a phosphorylated tyrosine side chain in a specific amino acid sequence context, as shown in Figure 3–39B. The amino acids located at the binding site for the phosphorylated polypeptide have been the slowest to change during the long evolutionary process that produced the large SH2 family of peptide recognition domains. Because mutation is a random process, this result is attributed to the preferential elimination during evolution of all organisms whose SH2 domains became altered in a way that inactivated the SH2-binding site, thereby destroying the function of the SH2 domain.

Figure 3–39 The evolutionary trace method applied to the SH2 domain. (A) Front and back views of a space-filling model of the SH2 domain, with evolutionarily conserved amino acids on the protein surface colored *yellow*, and those more toward the protein interior colored *red*. (B) The structure of the SH2 domain with its bound polypeptide. Here, those amino acids located within 0.4 nm of the bound ligand are colored *blue*. The two key amino acids of the ligand are *yellow*, and the others are *purple*. Note the high degree of correspondance between (A) and (B). (Adapted from O. Lichtarge, H.R. Bourne and F.E. Cohen, *J. Mol. Biol.* 257:342–358, 1996. With permission from Elsevier.)



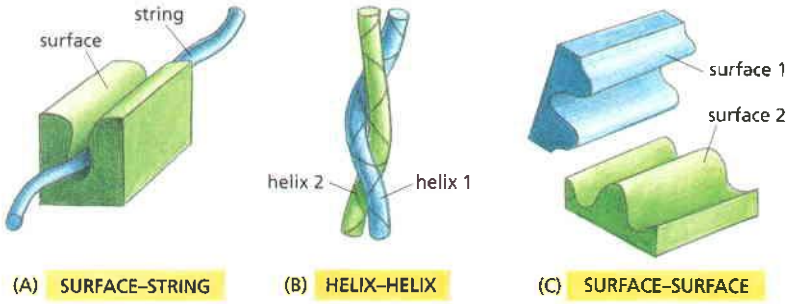


Figure 3–40 Three ways in which two proteins can bind to each other. Only the interacting parts of the two proteins are shown. (A) A rigid surface on one protein can bind to an extended loop of polypeptide chain (a "string") on a second protein. (B) Two α helices can bind together to form a coiled-coil. (C) Two complementary rigid surfaces often link two proteins together.

In this era of extensive genome sequencing, many new protein families have been discovered whose functions are unknown. Once a three-dimensional structure has been determined for one family member, evolutionary tracing allows biologists to determine binding sites for the members of that family, thereby helping to decipher protein function.

Proteins Bind to Other Proteins Through Several Types of Interfaces

Proteins can bind to other proteins in at least three ways. In many cases, a portion of the surface of one protein contacts an extended loop of polypeptide chain (a "string") on a second protein (**Figure 3–40**A). Such a surface–string interaction, for example, allows the SH2 domain to recognize a phosphorylated polypeptide loop on a second protein, as just described, and it also enables a protein kinase to recognize the proteins that it will phosphorylate (see below).

A second type of protein–protein interface forms when two α helices, one from each protein, pair together to form a coiled-coil (Figure 3–40B). This type of protein interface is found in several families of gene regulatory proteins, as discussed in Chapter 7.

The most common way for proteins to interact, however, is by the precise matching of one rigid surface with that of another (Figure 3–40C). Such interactions can be very tight, since a large number of weak bonds can form between two surfaces that match well. For the same reason, such surface–surface interactions can be extremely specific, enabling a protein to select just one partner from the many thousands of different proteins found in a cell.

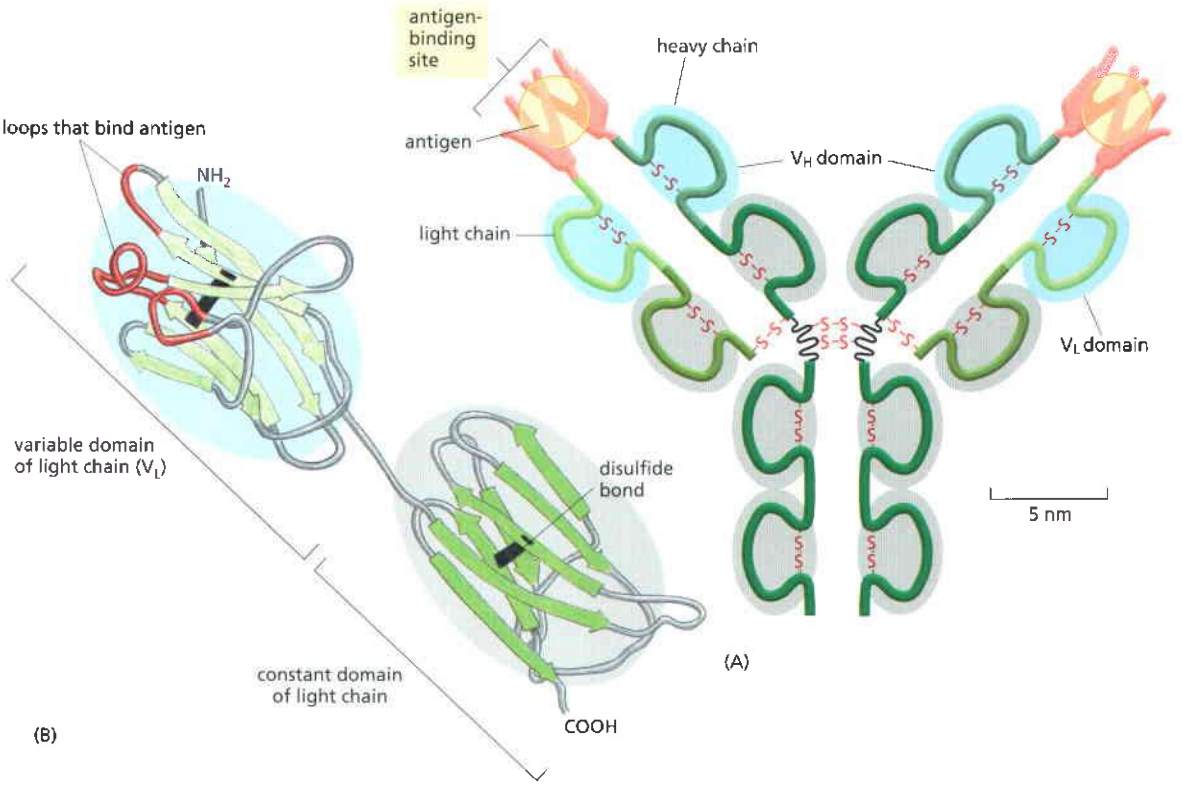
Antibody Binding Sites Are Especially Versatile <GCCG>

All proteins must bind to particular ligands to carry out their various functions. The antibody family is notable for its capacity for tight selective binding (discussed in detail in Chapter 25).

Antibodies, or immunoglobulins, are proteins produced by the immune system in response to foreign molecules, such as those on the surface of an invading microorganism. Each antibody binds tightly to a particular target molecule, thereby either inactivating the target molecule directly or marking it for destruction. An antibody recognizes its target (called an **antigen**) with remarkable specificity. Because there are potentially billions of different antigens that humans might encounter, we have to be able to produce billions of different antibodies.

Antibodies are Y-shaped molecules with two identical binding sites that are complementary to a small portion of the surface of the antigen molecule. A detailed examination of the antigen-binding sites of antibodies reveals that they are formed from several loops of polypeptide chain that protrude from the ends of a pair of closely juxtaposed protein domains (**Figure 3–41**). Different antibodies generate an enormous diversity of antigen-binding sites by changing only the length and amino acid sequence of these loops, without altering the basic protein structure.

Loops of this kind are ideal for grasping other molecules. They allow a large number of chemical groups to surround a ligand so that the protein can link to



it with many weak bonds. For this reason, loops often form the ligand-binding sites in proteins.

Figure 3–41 An antibody molecule. (A) A typical antibody molecule is Y-shaped and has two identical binding sites for its antigen, one on each arm of the Y. The protein is composed of four polypeptide chains (two identical heavy chains and two identical and smaller light chains) held together by disulfide bonds. Each chain is made up of several different immunoglobulin domains, here shaded either *blue* or *gray*. The antigen-binding site is formed where a heavy-chain variable domain (V_H) and a light-chain variable domain (V_L) come close together. These are the domains that differ most in their sequence and structure in different antibodies. Each domain at the end of the two arms of the antibody molecule forms loops that bind to the antigen. In (B) we can see these fingerlike loops *(red)* contributed by the V_L domain.

The Equilibrium Constant Measures Binding Strength

Molecules in the cell encounter each other very frequently because of their continual random thermal movements. Colliding molecules with poorly matching surfaces form few noncovalent bonds with one another, and the two molecules dissociate as rapidly as they come together. At the other extreme, when many noncovalent bonds form between two colliding molecules, the association can persist for a very long time (**Figure 3–42**). Strong interactions occur in cells whenever a biological function requires that molecules remain associated for a long time—for example, when a group of RNA and protein molecules come together to make a subcellular structure such as a ribosome.

We can measure the strength with which any two molecules bind to each other. As an example, consider a population of identical antibody molecules that suddenly encounters a population of ligands diffusing in the fluid surrounding them. At frequent intervals, one of the ligand molecules will bump into the binding site of an antibody and form an antibody–ligand complex. The population of antibody–ligand complexes will therefore increase, but not without limit: over time, a second process, in which individual complexes break apart because of thermally induced motion, will become increasingly important. Eventually, any population of antibody molecules and ligands will reach a steady state, or equilibrium, in which the number of binding (association) events per second is precisely equal to the number of "unbinding" (dissociation) events (see Figure 2–52).

From the concentrations of the ligand, antibody, and antibody–ligand complex at equilibrium, we can calculate a convenient measure—the **equilibrium constant** ***(K)***—of the strength of binding (**Figure 3–43**A). The equilibrium constant for a reaction in which two molecules (A and B) bind to each other to form a complex (AB) has units of liters/mole, and half of the binding sites will be occupied by ligand when that ligand's concentration (in moles/liter) reaches a value that is equal to $1/K$. This equilibrium constant is larger the greater the binding strength, and it is a direct measure of the free-energy difference

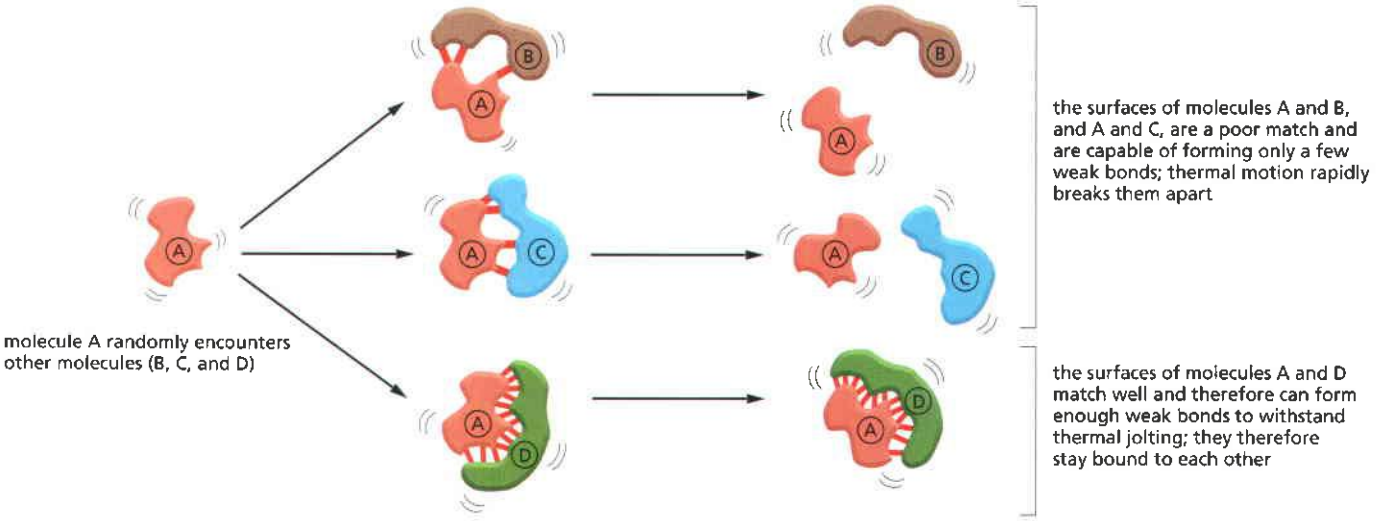


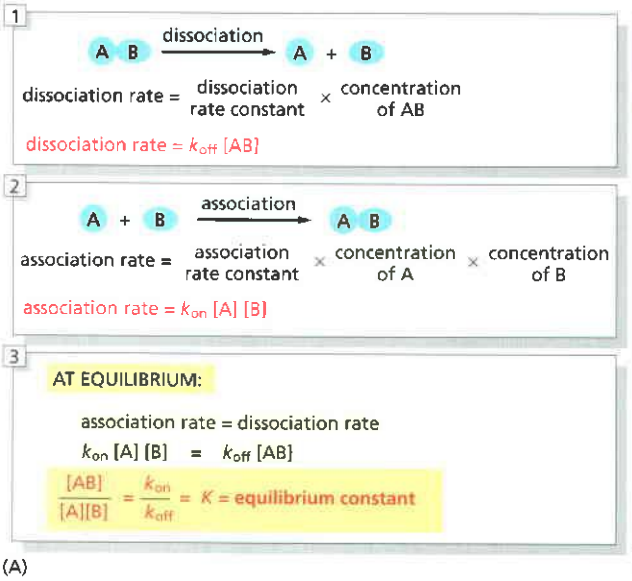
Figure 3–42 How noncovalent bonds mediate interactions between macromolecules.

between the bound and free states (Figure 3–43B and C). Even a change of a few noncovalent bonds can have a striking effect on a binding interaction, as shown by the example in Figure 3–44. (Note that the equilibrium constant, as defined here is also known as the association or affinity constant, K_a.)

We have used the case of an antibody binding to its ligand to illustrate the effect of binding strength on the equilibrium state, but the same principles apply to any molecule and its ligand. Many proteins are enzymes, which, as we now discuss, first bind to their ligands and then catalyze the breakage or formation of covalent bonds in these molecules.

Enzymes Are Powerful and Highly Specific Catalysts

Many proteins can perform their function simply by binding to another molecule. An actin molecule, for example, need only associate with other actin



The relationship between free-energy differences and equilibrium constants (37°C)

equilibrium constant $\frac{[AB]}{[A][B]} = K$ (liters/mole)	free-energy difference of AB minus free energy of A + B (kcal/mole)	free-energy difference of AB minus free energy of A + B (kJ/mole)
1	0	0
10	−1.4	−5.9
10^2	−2.8	−11.9
10^3	−4.3	−17.8
10^4	−5.7	−23.7
10^5	−7.1	−29.7
10^6	−8.5	−35.6
10^7	−9.9	−41.5
10^8	−11.3	−47.4
10^9	−12.8	−53.4
10^{10}	−14.2	−59.4
10^{11}	−15.6	−65.3

(B)

Although joules and kilojoules (1000 joules) are standard units of energy, cell biologists usually refer to free energy values in terms of calories and kilocalories.

One kilocalorie (kcal) is equal to 4.184 kilojoules (kJ).

The relationship between the free-energy change, ΔG, and the equilibrium constant is

$\Delta G = -0.00458\ T \log K$

where ΔG is in kilocalories and T is the absolute temperature in Kelvins (310 K = 37°C).

(C)

Figure 3–43 Relating binding energies to the equilibrium constant for an association reaction. (A) The equilibrium between molecules A and B and the complex AB is maintained by a balance between the two opposing reactions shown in panels 1 and 2. Molecules A and B must collide if they are to react, and the association rate is therefore proportional to the product of their individual concentrations [A] × [B]. (Square brackets indicate concentration.) As shown in panel 3, the ratio of the rate constants for the association and the dissociation reactions is equal to the equilibrium constant (K) for the reaction. (B) The equilibrium constant in panel 3 is that for the reaction A + B ⇌ AB, and the larger its value, the stronger the binding between A and B. Note that for every 1.41 kcal/mole (5.91 kJ/mole) decrease in free energy the equilibrium constant increases by a factor of 10 at 37°C.

The equilibrium constant here has units of liters/mole: for simple binding interactions it is also called the *affinity constant* or *association constant*, denoted K_a. The reciprocal of K_a is called the dissociation constant, K_d (in units of moles/liter).

molecules to form a filament. There are other proteins, however, for which ligand binding is only a necessary first step in their function. This is the case for the large and very important class of proteins called **enzymes**. As described in Chapter 2, enzymes are remarkable molecules that determine all the chemical transformations that make and break covalent bonds in cells. They bind to one or more ligands, called **substrates**, and convert them into one or more chemically modified *products*, doing this over and over again with amazing rapidity. Enzymes speed up reactions, often by a factor of a million or more, without themselves being changed—that is, they act as **catalysts** that permit cells to make or break covalent bonds in a controlled way. It is the catalysis of organized sets of chemical reactions by enzymes that creates and maintains the cell, making life possible.

We can group enzymes into functional classes that perform similar chemical reactions (Table 3–1). Each type of enzyme within such a class is highly specific, catalyzing only a single type of reaction. Thus, *hexokinase* adds a phosphate group to D-glucose but ignores its optical isomer L-glucose; the blood-clotting enzyme *thrombin* cuts one type of blood protein between a particular arginine and its adjacent glycine and nowhere else, and so on. As discussed in detail in Chapter 2, enzymes work in teams, with the product of one enzyme becoming the substrate for the next. The result is an elaborate network of metabolic pathways that provides the cell with energy and generates the many large and small molecules that the cell needs (see Figure 2–35).

Consider 1000 molecules of A and 1000 molecules of B in a eucaryotic cell. The concentration of both will be about 10^{-9} M.

If the equilibrium constant (K) for A + B ⇌ AB is 10^{10}, then one can calculate that at equilibrium there will be

270	270	730
A molecules	B molecules	AB molecules

If the equilibrium constant is a little weaker at 10^{8}, which represents a loss of 2.8 kcal/mole of binding energy from the example above, or 2–3 fewer hydrogen bonds, then there will be

915	915	85
A molecules	B molecules	AB molecules

Figure 3–44 Small changes in the number of weak bonds can have drastic effects on a binding interaction. This example illustrates the dramatic effect of the presence or absence of a few weak noncovalent bonds in a biological context.

Substrate Binding Is the First Step in Enzyme Catalysis

For a protein that catalyzes a chemical reaction (an enzyme), the binding of each substrate molecule to the protein is an essential prelude. In the simplest case, if we denote the enzyme by E, the substrate by S, and the product by P, the basic reaction path is E + S → ES → EP → E + P. From this reaction path, we see that there is a limit to the amount of substrate that a single enzyme molecule can process in a given time. An increase in the concentration of substrate also increases the rate at which product is formed, up to a maximum value (**Figure 3–45**). At that point the enzyme molecule is saturated with substrate, and the rate of reaction (V_{max}) depends only on how rapidly the enzyme can process the substrate molecule. This maximum rate divided by the enzyme concentration is

Table 3–1 Some Common Types of Enzymes

ENZYME	REACTION CATALYZED
Hydrolases	general term for enzymes that catalyze a hydrolytic cleavage reaction; *nucleases* and *proteases* are more specific names for subclasses of these enzymes.
Nucleases	break down nucleic acids by hydrolyzing bonds between nucleotides.
Proteases	break down proteins by hydrolyzing bonds between amino acids.
Synthases	synthesize molecules in anabolic reactions by condensing two smaller molecules together.
Isomerases	catalyze the rearrangement of bonds within a single molecule.
Polymerases	catalyze polymerization reactions such as the synthesis of DNA and RNA.
Kinases	catalyze the addition of phosphate groups to molecules. Protein kinases are an important group of kinases that attach phosphate groups to proteins.
Phosphatases	catalyze the hydrolytic removal of a phosphate group from a molecule.
Oxido-Reductases	general name for enzymes that catalyze reactions in which one molecule is oxidized while the other is reduced. Enzymes of this type are often more specifically named either *oxidases, reductases,* or *dehydrogenases.*
ATPases	hydrolyze ATP. Many proteins with a wide range of roles have an energy-harnessing ATPase activity as part of their function, for example, motor proteins such as *myosin* and membrane transport proteins such as the *sodium–potassium pump.*

Enzyme names typically end in "-ase," with the exception of some enzymes, such as pepsin, trypsin, thrombin and lysozyme that were discovered and named before the convention became generally accepted at the end of the nineteenth century. The common name of an enzyme usually indicates the substrate and the nature of the reaction catalyzed. For example, citrate synthase catalyzes the synthesis of citrate by a reaction between acetyl CoA and oxaloacetate.

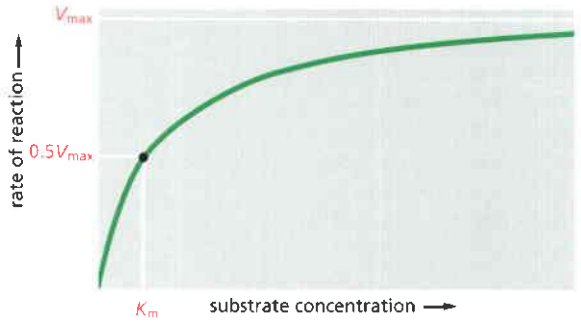


Figure 3–45 Enzyme kinetics. The rate of an enzyme reaction *(V)* increases as the substrate concentration increases until a maximum value (V_{max}) is reached. At this point all substrate-binding sites on the enzyme molecules are fully occupied, and the rate of reaction is limited by the rate of the catalytic process on the enzyme surface. For most enzymes, the concentration of substrate (K_m) at which the reaction rate is half-maximal (*black dot*) is a measure of how tightly the substrate is bound, with a large value of K_m corresponding to weak binding.

called the *turnover number*. The turnover number is often about 1000 substrate molecules processed per second per enzyme molecule, although turnover numbers between 1 and 10,000 are known.

The other kinetic parameter frequently used to characterize an enzyme is its K_m, the concentration of substrate that allows the reaction to proceed at one-half its maximum rate (0.5 V_{max}) (see Figure 3–45). A *low* K_m value means that the enzyme reaches its maximum catalytic rate at a *low concentration* of substrate and generally indicates that the enzyme binds to its substrate very tightly, whereas a *high* K_m value corresponds to weak binding. The methods used to characterize enzymes in this way are explained in **Panel 3–3** (pp. 162–163).

Enzymes Speed Reactions by Selectively Stabilizing Transition States

Enzymes achieve extremely high rates of chemical reaction—rates that are far higher than for any synthetic catalysts. There are several reasons for this efficiency. First, the enzyme increases the local concentration of substrate molecules at the catalytic site and holds all the appropriate atoms in the correct orientation for the reaction that is to follow. More importantly, however, some of the binding energy contributes directly to the catalysis. Substrate molecules must pass through a series of intermediate states of altered geometry and electron distribution before they form the ultimate products of the reaction. The free energy required to attain the most unstable **transition state** is called the *activation energy* for the reaction, and it is the major determinant of the reaction rate. Enzymes have a much higher affinity for the transition state of the substrate than they have for the stable form. Because this tight binding greatly lowers the energies of the transition state, the enzyme greatly accelerates a particular reaction by lowering the activation energy that is required (**Figure 3–46**).

By intentionally producing antibodies that act like enzymes, we can demonstrate that stabilizing a transition state can greatly increase a reaction rate. Consider, for example, the hydrolysis of an amide bond, which is similar to the peptide bond that joins two adjacent amino acids in a protein. In an aqueous solution, an amide bond hydrolyzes very slowly by the mechanism shown in **Figure 3–47**A. In the central intermediate, or transition state, the carbonyl carbon is bonded to four atoms arranged at the corners of a tetrahedron. By generating monoclonal antibodies that bind tightly to a stable analog of this very unstable *tetrahedral intermediate*, one can obtain an antibody that functions like an enzyme (Figure 3–47B). Because this *catalytic antibody* binds to and stabilizes the tetrahedral intermediate, it increases the spontaneous rate of amide-bond hydrolysis more than 10,000-fold.

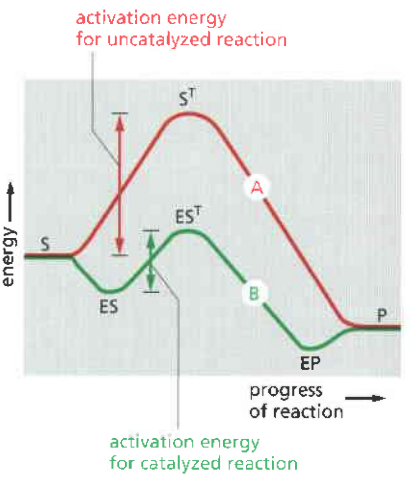


Figure 3–46 Enzymatic acceleration of chemical reactions by decreasing the activation energy. Often both the uncatalyzed reaction (A) and the enzyme-catalyzed reaction (B) can go through several transition states. It is the transition state with the highest energy (S^T and ES^T) that determines the activation energy and limits the rate of the reaction. (S = substrate; P = product of the reaction; ES = enzyme–substrate complex; EP = enzyme–product complex.)

Enzymes Can Use Simultaneous Acid and Base Catalysis

Figure 3–48 compares the spontaneous reaction rates and the corresponding enzyme-catalyzed rates for five enzymes. Rate accelerations range from 10^9 to 10^{23}. Clearly, enzymes are much better catalysts than catalytic antibodies.

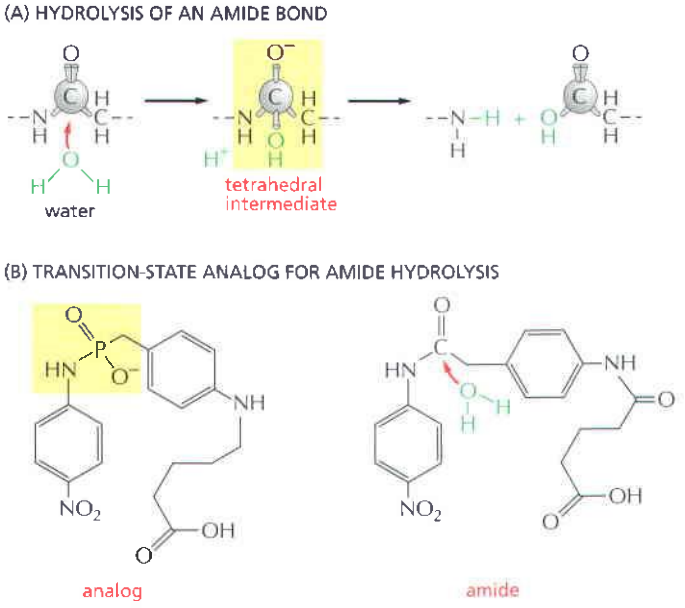


Figure 3–47 Catalytic antibodies. The stabilization of a transition state by an antibody creates an enzyme. (A) The reaction path for the hydrolysis of an amide bond goes through a tetrahedral intermediate, the high-energy transition state for the reaction. (B) The molecule on the left was covalently linked to a protein and used as an antigen to generate an antibody that binds tightly to the region of the molecule shown in *yellow*. Because this antibody also bound tightly to the transition state in (A), it was found to function as an enzyme that efficiently catalyzed the hydrolysis of the amide bond in the molecule on the right.

Enzymes not only bind tightly to a transition state, they also contain precisely positioned atoms that alter the electron distributions in those atoms that participate directly in the making and breaking of covalent bonds. Peptide bonds, for example, can be hydrolyzed in the absence of an enzyme by exposing a polypeptide to either a strong acid or a strong base, as illustrated in **Figure 3–49**. Enzymes are unique, however, in being able to use acid and base catalysis simultaneously, since the rigid framework of the protein binds the acidic and basic residues and prevents them from combining with each other (as they would do in solution) (Figure 3–49D).

The fit between an enzyme and its substrate needs to be precise. A small change introduced by genetic engineering in the active site of an enzyme can have a profound effect. Replacing a glutamic acid with an aspartic acid in one enzyme, for example, shifts the position of the catalytic carboxylate ion by only 1 Å (about the radius of a hydrogen atom); yet this is enough to decrease the activity of the enzyme a thousandfold.

Lysozyme Illustrates How an Enzyme Works <AGCA>

To demonstrate how enzymes catalyze chemical reactions, we examine an enzyme that acts as a natural antibiotic in egg white, saliva, tears, and other secretions. **Lysozyme** catalyzes the cutting of polysaccharide chains in the cell walls of bacteria. Because the bacterial cell is under pressure from osmotic forces, cutting even a small number of polysaccharide chains causes the cell wall to rupture and the cell to burst. Lysozyme is a relatively small and stable protein

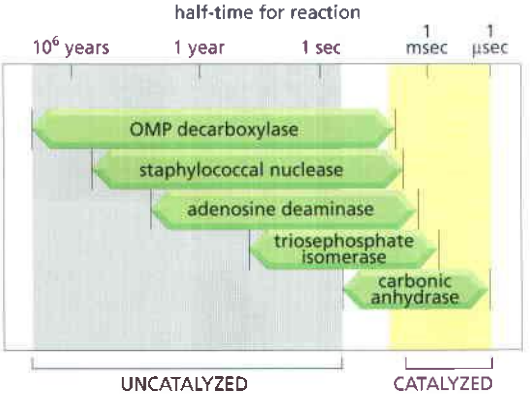


Figure 3–48 The rate accelerations caused by five different enzymes. (Adapted from A. Radzicka and R. Wolfenden, *Science* 267:90–93, 1995. With permission from AAAS.)

WHY ANALYZE THE KINETICS OF ENZYMES?

Enzymes are the most selective and powerful catalysts known. An understanding of their detailed mechanisms provides a critical tool for the discovery of new drugs, for the large-scale industrial synthesis of useful chemicals, and for appreciating the chemistry of cells and organisms. A detailed study of the rates of the chemical reactions that are catalyzed by a purified enzyme—more specifically how these rates change with changes in conditions such as the concentrations of substrates, products, inhibitors, and regulatory ligands—allows biochemists to figure out exactly how each enzyme works. For example, this is the way that the ATP-producing reactions of glycolysis, shown previously in Figure 2–72, were deciphered—allowing us to appreciate the rationale for this critical enzymatic pathway.

In this Panel, we introduce the important field of enzyme kinetics, which has been indispensable for deriving much of the detailed knowledge that we now have about cell chemistry.

STEADY-STATE ENZYME KINETICS

Many enzymes have only one substrate, which they bind and then process to produce products according to the scheme outlined in Figure 3–50A. In this case, the reaction is written as

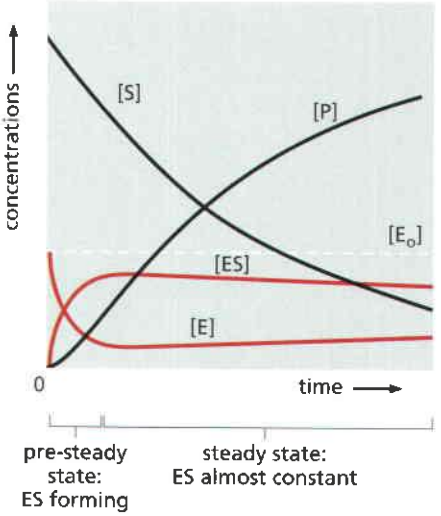
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

Here we have assumed that the reverse reaction, in which E + P recombine to form EP and then ES, occurs so rarely that we can ignore it. In this case, EP need not be represented, and we can express the rate of the reaction — known as its velocity, V, as

$$V = k_{cat}\,[ES]$$

where [ES] is the concentration of the enzyme–substrate complex, and k_{cat} is the turnover number, a rate constant that has a value equal to the number of substrate molecules processed per enzyme molecule each second.

But how does the value of [ES] relate to the concentrations that we know directly, which are the total concentration of the enzyme, $[E_o]$, and the concentration of the substrate, [S]? When enzyme and substrate are first mixed, the concentration [ES] will rise rapidly from zero to a so-called steady-state level, as illustrated below.



At this steady state, [ES] is nearly constant, so that

$$\text{rate of ES breakdown } k_{-1}\,[ES] + k_{cat}\,[ES] = \text{rate of ES formation } k_1\,[E][S]$$

or, since the concentration of the free enzyme, [E], is equal to $[E_o] - [ES]$,

$$[ES] = \left(\frac{k_1}{k_{-1} + k_{cat}}\right)[E][S] = \left(\frac{k_1}{k_{-1} + k_{cat}}\right)\left([E_o] - [ES]\right)[S]$$

Rearranging, and defining the constant K_m as

$$\frac{k_{-1} + k_{cat}}{k_1}$$

we get

$$[ES] = \frac{[E_o][S]}{K_m + [S]}$$

or, remembering that $V = k_{cat}\,[ES]$, we obtain the famous Michaelis–Menten equation

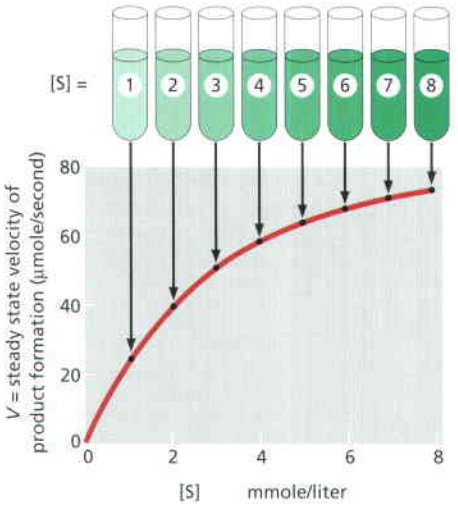
$$V = \frac{k_{cat}\,[E_o][S]}{K_m + [S]}$$

As [S] is increased to higher and higher levels, essentially all of the enzyme will be bound to substrate at steady state; at this point, a maximum rate of reaction, V_{max}, will be reached where $V = V_{max} = k_{cat}\,[E_o]$. Thus, it is convenient to rewrite the Michaelis–Menten equation as

$$V = \frac{V_{max}\,[S]}{K_m + [S]}$$

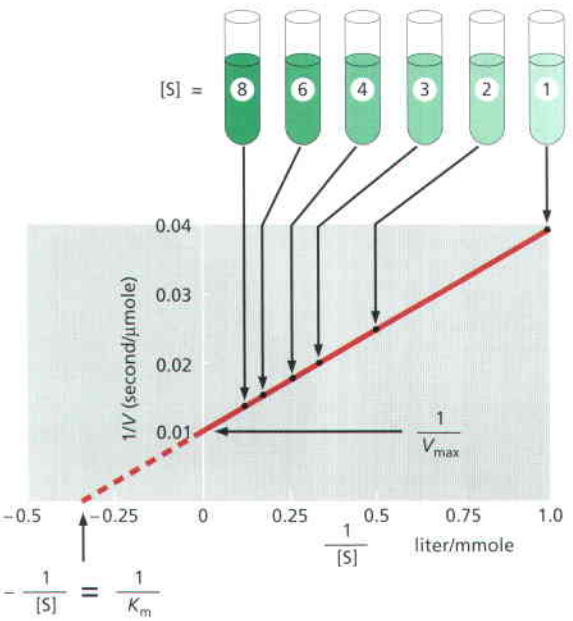
THE DOUBLE-RECIPROCAL PLOT

A typical plot of V versus [S] for an enzyme that follows Michaelis–Menten kinetics is shown below. From this plot, neither the value of V_{max} nor of K_m is immediately clear.



To obtain V_{max} and K_m from such data, a double-reciprocal plot is often used, in which the Michaelis–Menten equation has merely been rearranged, so that $1/V$ can be plotted versus $1/[S]$.

$$1/V = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{[S]}\right) + 1/V_{max}$$



THE SIGNIFICANCE OF K_m, k_{cat}, and k_{cat}/K_m

As described in the text, K_m is an approximate measure of substrate affinity for the enzyme: it is numerically equal to the concentration of [S] at $V = 0.5\ V_{max}$. In general, a lower value of K_m means tighter substrate binding. In fact, for those cases where k_{cat} is much smaller than k_{-1}, the K_m will be equal to K_d, the dissociation constant for substrate binding to the enzyme ($K_d = 1/K_a$).

We have seen that k_{cat} is the turnover number for the enzyme. At very low substrate concentrations, where $[S] << K_m$, most of the enzyme is free. Thus we can think of $[E] = [E_o]$, so that the Michaelis–Menten equation becomes $V = k_{cat}/K_m\ [E][S]$. Thus, the ratio k_{cat}/K_m is equivalent to the rate constant for the reaction between free enzyme and free substrate.

A comparison of k_{cat}/K_m for the same enzyme with different substrates, or for two enzymes with their different substrates, is widely used as a measure of enzyme effectiveness.

For simplicity, in this Panel we have discussed enzymes that have only one substrate, such as the lysozyme enzyme described in the text (see p. 164). Most enzymes have two substrates, one of which is often an active carrier molecule—such as NADH or ATP.

A similar, but more complex, analysis is used to determine the kinetics of such enzymes—allowing the order of substrate binding and the presence of covalent intermediates along the pathway to be revealed.

SOME ENZYMES ARE DIFFUSION LIMITED

The values of k_{cat}, K_m, and k_{cat}/K_m for some selected enzymes are given below:

enzyme	substrate	k_{cat} (sec^{-1})	K_m (M)	k_{at}/K_m ($sec^{-1}M^{-1}$)
acetylcholinesterase	acetylcholine	$1.4x10^4$	$9x10^{-5}$	$1.6x10^8$
catalase	H_2O_2	$4x10^7$	1	$4x10^7$
fumarase	fumarate	$8x10^2$	$5x10^{-6}$	$1.6x10^8$

Because an enzyme and its substrate must collide before they can react, k_{cat}/K_m has a maximum possible value that is limited by collision rates. If every collision forms an enzyme–substrate complex, one can calculate from diffusion theory that k_{cat}/K_m will be between 10^8 and 10^9 $sec^{-1}M^{-1}$, in the case where all subsequent steps proceed immediately. Thus, it is claimed that enzymes like acetylcholinesterase and fumarase are "perfect enzymes," each enzyme having evolved to the point where nearly every collision with its substrate converts the substrate to a product.

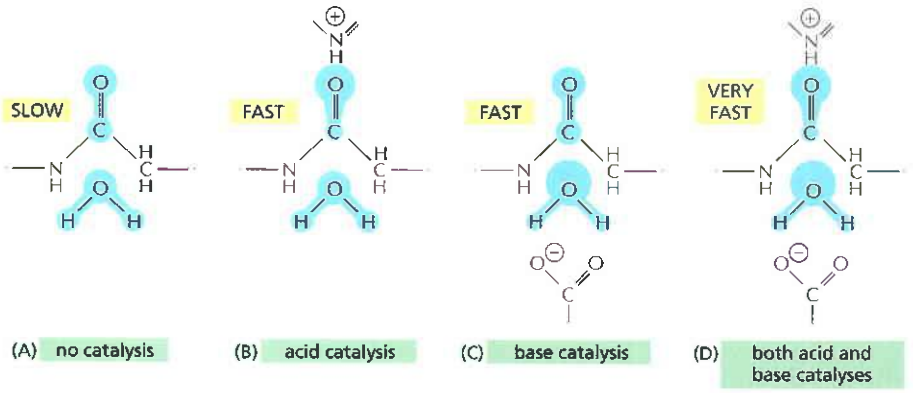


Figure 3–49 Acid catalysis and base catalysis. (A) The start of the uncatalyzed reaction shown in Figure 3–47A, with *blue* indicating electron distribution in the water and carbonyl bonds. (B) An acid likes to donate a proton (H^+) to other atoms. By pairing with the carbonyl oxygen, an acid causes electrons to move away from the carbonyl carbon, making this atom much more attractive to the electronegative oxygen of an attacking water molecule. (C) A base likes to take up H^+. By pairing with a hydrogen of the attacking water molecule, a base causes electrons to move toward the water oxygen, making it a better attacking group for the carbonyl carbon. (D) By having appropriately positioned atoms on its surface, an enzyme can perform both acid catalysis and base catalysis at the same time.

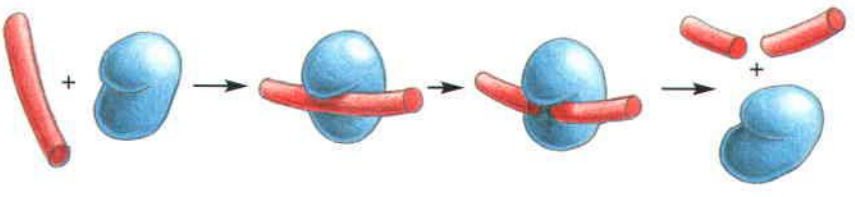
that can be easily isolated in large quantities. For these reasons, it has been intensively studied, and it was the first enzyme to have its structure worked out in atomic detail by x-ray crystallography.

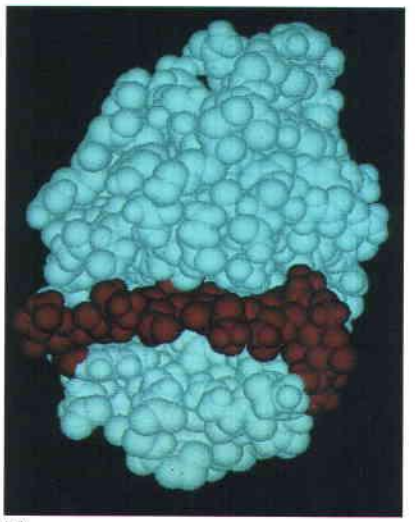
The reaction that lysozyme catalyzes is a hydrolysis: it adds a molecule of water to a single bond between two adjacent sugar groups in the polysaccharide chain, thereby causing the bond to break (see Figure 2–19). The reaction is energetically favorable because the free energy of the severed polysaccharide chain is lower than the free energy of the intact chain. However, the pure polysaccharide can remain for years in water without being hydrolyzed to any detectable degree. This is because there is an energy barrier to the reaction, as discussed in Chapter 2 (see Figure 2–46). A colliding water molecule can break a bond linking two sugars only if the polysaccharide molecule is distorted into a particular shape—the transition state—in which the atoms around the bond have an altered geometry and electron distribution. Because of this distortion, random collisions must supply a very large activation energy for the reaction to take place. In an aqueous solution at room temperature, the energy of collisions almost never exceeds the activation energy. Consequently, hydrolysis occurs extremely slowly, if at all.

This situation changes drastically when the polysaccharide binds to lysozyme. The active site of lysozyme, because its substrate is a polymer, is a long groove that holds six linked sugars at the same time. As soon as the polysaccharide binds to form an enzyme–substrate complex, the enzyme cuts the polysaccharide by adding a water molecule across one of its sugar–sugar bonds. The product chains are then quickly released, freeing the enzyme for further cycles of reaction (**Figure 3–50**).

The chemistry of the binding of lysozyme to its substrate is the same as that for antibody binding to its antigen—the formation of multiple noncovalent

Figure 3–50 The reaction catalyzed by lysozyme. (A) The enzyme lysozyme (E) catalyzes the cutting of a polysaccharide chain, which is its substrate (S). The enzyme first binds to the chain to form an enzyme–substrate complex (ES) and then catalyzes the cleavage of a specific covalent bond in the backbone of the polysaccharide, forming an enzyme–product complex (EP) that rapidly dissociates. Release of the severed chain (the products P) leaves the enzyme free to act on another substrate molecule. (B) A space-filling model of the lysozyme molecule bound to a short length of polysaccharide chain before cleavage. (B, courtesy of Richard J. Feldmann.)




(B)

bonds. However, lysozyme holds its polysaccharide substrate in a particular way, so that it distorts one of the two sugars in the bond to be broken from its normal, most stable conformation. The bond to be broken is also held close to two amino acids with acidic side chains (a glutamic acid and an aspartic acid) within the active site.

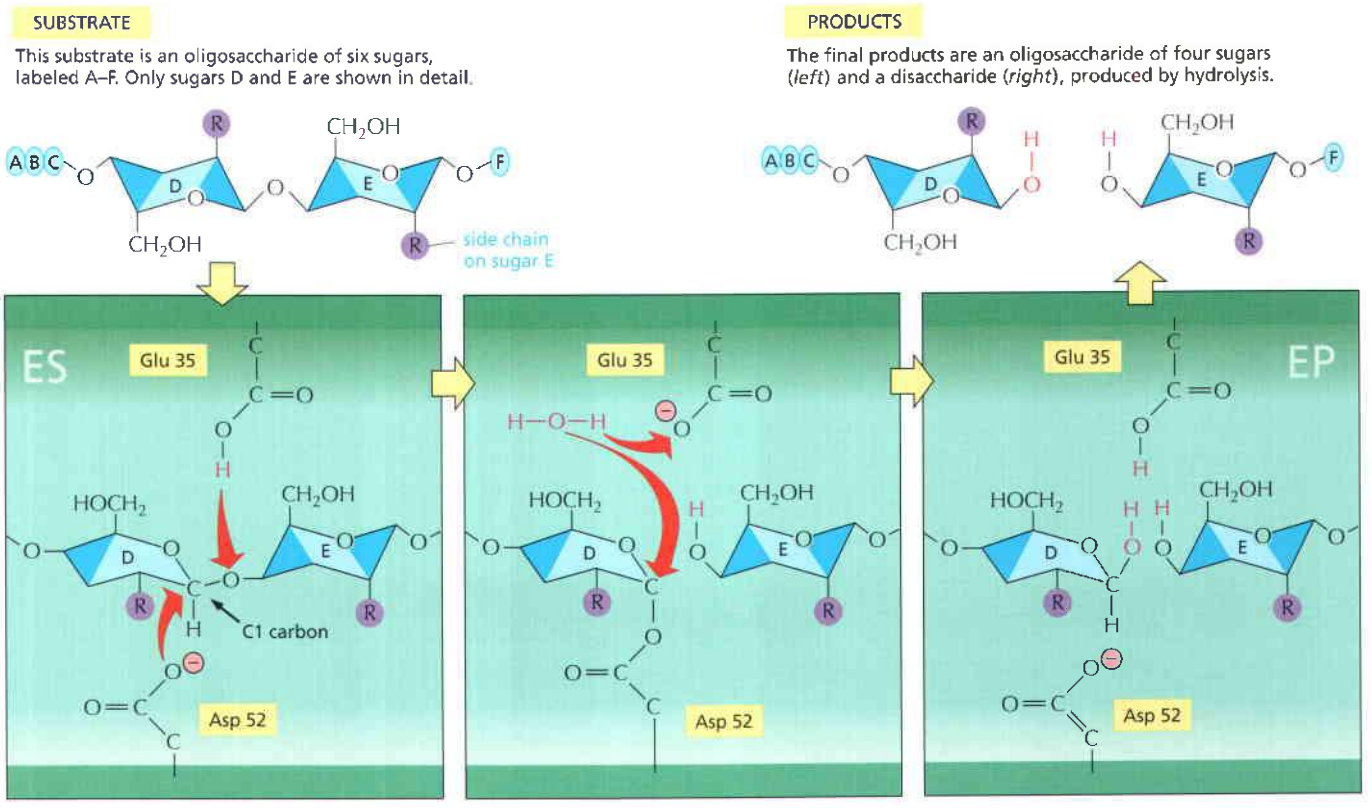
Conditions are thereby created in the microenvironment of the lysozyme active site that greatly reduce the activation energy necessary for the hydrolysis to take place. **Figure 3–51** shows three central steps in this enzymatically catalyzed reaction.

1. The enzyme stresses its bound substrate, so that the shape of one sugar more closely resembles the shape of high-energy transition states formed during the reaction.
2. The negatively charged aspartic acid reacts with the C1 carbon atom on the distorted sugar, and the glutamic acid donates its proton to the oxygen that links this sugar to its neighbor. This breaks the sugar–sugar bond and leaves the aspartic acid side chain covalently linked to the site of bond cleavage.
3. Aided by the negatively charged glutamic acid, a water molecule reacts with the C1 carbon atom, displacing the aspartic acid side chain and completing the process of hydrolysis.

The overall chemical reaction, from the initial binding of the polysaccharide on the surface of the enzyme through the final release of the severed chains, occurs many millions of times faster than it would in the absence of enzyme.

Other enzymes use similar mechanisms to lower activation energies and speed up the reactions they catalyze. In reactions involving two or more reactants, the active site also acts like a template, or mold, that brings the substrates together in the proper orientation for a reaction to occur between them (**Figure**

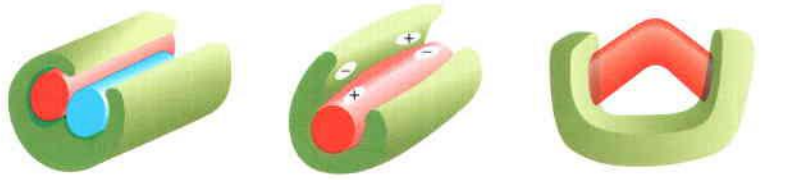
Figure 3–51 Events at the active site of lysozyme. <TGGT> The top left and top right drawings show the free substrate and the free products, respectively, whereas the other three drawings show the sequential events at the enzyme active site. Note the change in the conformation of sugar D in the enzyme–substrate complex; this shape change stabilizes the oxocarbenium ion-like transition states required for formation and hydrolysis of the covalent intermediate shown in the middle panel. It is also possible that a carbonium ion intermediate forms in step 2, as the covalent intermediate shown in the middle panel has been detected only with a synthetic substrate. (See D.J. Vocadlo et al., *Nature* 412:835–838, 2001.)



In the enzyme–substrate complex (ES), the enzyme forces sugar D into a strained conformation, with Glu 35 positioned to serve as an acid that attacks the adjacent sugar–sugar bond by donating a proton (H^+) to sugar E, and Asp 52 poised to attack the C1 carbon atom.

The Asp 52 has formed a covalent bond between the enzyme and the C1 carbon atom of sugar D. The Glu 35 then polarizes a water molecule (*red*), so that its oxygen can readily attack the C1 carbon atom and displace Asp 52.

The reaction of the water molecule (*red*) completes the hydrolysis and returns the enzyme to its initial state, forming the final enzyme–product complex (EP).



(A) enzyme binds to two substrate molecules and orients them precisely to encourage a reaction to occur between them

(B) binding of substrate to enzyme rearranges electrons in the substrate, creating partial negative and positive charges that favor a reaction

(C) enzyme strains the bound substrate molecule, forcing it toward a transition state to favor a reaction

Figure 3–52 Some general strategies of enzyme catalysis. (A) Holding substrates together in a precise alignment. (B) Charge stabilization of reaction intermediates. (C) Applying forces that distort bonds in the substrate to increase the rate of a particular reaction.

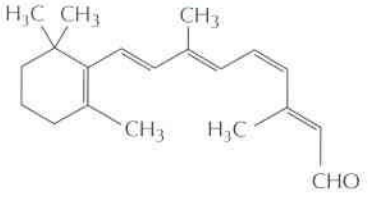
3–52A). As we saw for lysozyme, the active site of an enzyme contains precisely positioned atoms that speed up a reaction by using charged groups to alter the distribution of electrons in the substrates (Figure 3–52B). In addition, when a substrate binds to an enzyme, bonds in the substrate often bend, changing the substrate shape. These changes, along with mechanical forces, drive a substrate toward a particular transition state (Figure 3–52C). Finally, like lysozyme, many enzymes participate intimately in the reaction by briefly forming a covalent bond between the substrate and a side chain of the enzyme. Subsequent steps in the reaction restore the side chain to its original state, so that the enzyme remains unchanged after the reaction (see also Figure 2–72).

Tightly Bound Small Molecules Add Extra Functions to Proteins

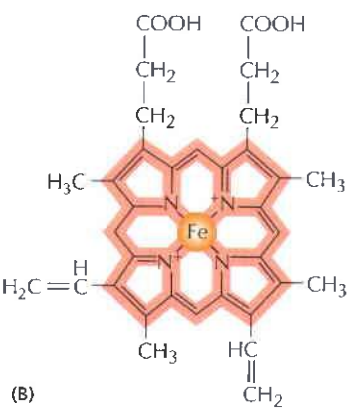
Although we have emphasized the versatility of proteins as chains of amino acids that perform different functions, there are many instances in which the amino acids by themselves are not enough. Just as humans employ tools to enhance and extend the capabilities of their hands, proteins often use small nonprotein molecules to perform functions that would be difficult or impossible to do with amino acids alone. Thus, the signal receptor protein *rhodopsin*, which is made by the photoreceptor cells in the retina, detects light by means of a small molecule, *retinal*, embedded in the protein (**Figure 3–53**A). Retinal changes its shape when it absorbs a photon of light, and this change causes the protein to trigger a cascade of enzymatic reactions that eventually lead to an electrical signal being carried to the brain.

Another example of a protein that contains a nonprotein portion is hemoglobin (see Figure 3–22). A molecule of hemoglobin carries four *heme* groups, ring-shaped molecules each with a single central iron atom (Figure 3–53B). Heme gives hemoglobin (and blood) its red color. By binding reversibly to oxygen gas through its iron atom, heme enables hemoglobin to pick up oxygen in the lungs and release it in the tissues.

Sometimes these small molecules are attached covalently and permanently to their protein, thereby becoming an integral part of the protein molecule itself. We shall see in Chapter 10 that proteins are often anchored to cell membranes through covalently attached lipid molecules. And membrane proteins exposed



(A)



(B)

Figure 3–53 Retinal and heme. (A) The structure of retinal, the light-sensitive molecule attached to rhodopsin in the eye. (B) The structure of a heme group. The carbon-containing heme ring is *red* and the iron atom at its center is *orange*. A heme group is tightly bound to each of the four polypeptide chains in hemoglobin, the oxygen-carrying protein whose structure is shown in Figure 3–22.

Table 3–2 Many Vitamins Provide Critical Coenzymes for Human Cells

VITAMIN	COENZYME	ENZYME-CATALYZED REACTIONS REQUIRING THESE COENZYMES
Thiamine (vitamin B_1)	thiamine pyrophosphate	activation and transfer of aldehydes
Riboflavin (vitamin B_2)	FADH	oxidation–reduction
Niacin	NADH, NADPH	oxidation–reduction
Pantothenic acid	coenzyme A	acyl group activation and transfer
Pyridoxine	pyridoxal phosphate	amino acid activation; also glycogen phosphorylase
Biotin	biotin	CO_2 activation and transfer
Lipoic acid	lipoamide	acyl group activation; oxidation–reduction
Folic acid	tetrahydrofolate	activation and transfer of single carbon groups
Vitamin B_{12}	cobalamin coenzymes	isomerization and methyl group transfers

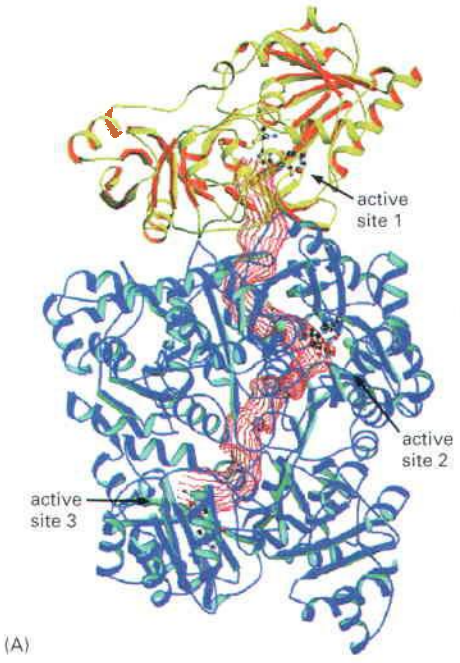
on the surface of the cell, as well as proteins secreted outside the cell, are often modified by the covalent addition of sugars and oligosaccharides.

Enzymes frequently have a small molecule or metal atom tightly associated with their active site that assists with their catalytic function. *Carboxypeptidase*, for example, an enzyme that cuts polypeptide chains, carries a tightly bound zinc ion in its active site. During the cleavage of a peptide bond by carboxypeptidase, the zinc ion forms a transient bond with one of the substrate atoms, thereby assisting the hydrolysis reaction. In other enzymes, a small organic molecule serves a similar purpose. Such organic molecules are often referred to as **coenzymes**. An example is *biotin*, which is found in enzymes that transfer a carboxylate group ($-COO^-$) from one molecule to another (see Figure 2–63). Biotin participates in these reactions by forming a transient covalent bond to the $-COO^-$ group to be transferred, being better suited to this function than any of the amino acids used to make proteins. Because it cannot be synthesized by humans, and must therefore be supplied in small quantities in our diet, biotin is a *vitamin*. Many other coenzymes are produced from vitamins (**Table 3–2**). Vitamins are also needed to make other types of small molecules that are essential components of our proteins; vitamin A, for example, is needed in the diet to make retinal, the light-sensitive part of rhodopsin.

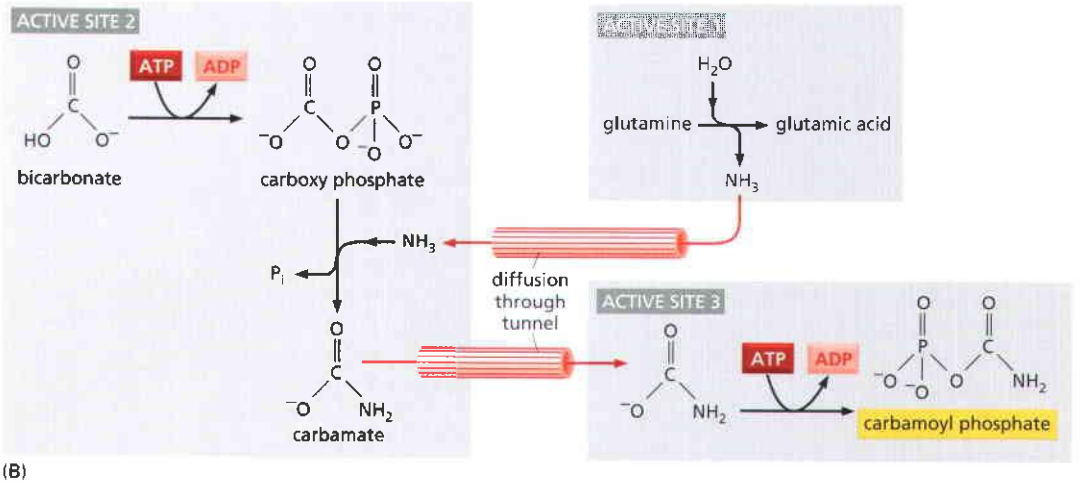
Molecular Tunnels Channel Substrates in Enzymes with Multiple Catalytic Sites

Some of the chemical reactions catalyzed by enzymes in cells produce intermediates that are either very unstable or that could readily diffuse out of the cell through the plasma membrane if released into the cytosol. To preserve these intermediates, enzymes have evolved *molecular tunnels* that connect two or more active sites, allowing the intermediate to be rapidly processed to a final product—without ever leaving the enzyme.

Consider, for example, the enzyme carbamoyl phosphate synthetase, which uses ammonia derived from glutamine plus two molecules of ATP to convert bicarbonate (HCO_3^-) to carbamoyl phosphate—an important intermediate in several metabolic pathways (**Figure 3–54**). This enzyme contains three widely separated active sites that are connected to each other by a tunnel. The reaction starts at active site 2, located in the middle of the tunnel, where ATP is used to phosphorylate (add a phosphate group to) bicarbonate, forming carboxy phosphate. This event triggers the hydrolysis of glutamine to glutamic acid at active site 1, releasing ammonia into the tunnel. The ammonia immediately diffuses through the first half of the tunnel to active site 2, where it reacts with the carboxyphosphate to form carbamate. This unstable intermediate then diffuses through the second half of the tunnel to active site 3, where it is phosphorylated by ATP to the final product, carbamoyl phosphate.



(A)



(B)

Figure 3–54 The tunneling of reaction intermediates in the enzyme carbamoyl phosphate synthetase. (A) Diagram of the structure of the enzyme, in which a *red* ribbon has been used to outline the tunnel on the inside of the protein connecting its three active sites. The small and large subunits of this dimeric enzyme are color coded *yellow* and *blue*, respectively. (B) The path of the reaction. As indicated, active site 1 produces ammonia, which diffuses through the tunnel to active site 2, where it combines with carboxy phosphate to form carbamate. This highly unstable intermediate then diffuses through the tunnel to active site 3, where it is phosphorylated by ATP to produce the final product, carbamoyl phosphate. (A, modified from F.M. Raushel, J.B. Thoden, and H.M. Holden, *Acc. Chem. Res.* 36:539–548, 2003. With permission from American Chemical Society.)

Several other well characterized enzymes contain similar molecular tunnels. Ammonia, a readily diffusable intermediate that might otherwise be lost from the cell, is the substrate most frequently channeled in the examples thus far known.

Multienzyme Complexes Help to Increase the Rate of Cell Metabolism

The efficiency of enzymes in accelerating chemical reactions is crucial to the maintenance of life. Cells, in effect, must race against the unavoidable processes of decay, which—if left unattended—cause macromolecules to run downhill toward greater and greater disorder. If the rates of desirable reactions were not greater than the rates of competing side reactions, a cell would soon die. We can get some idea of the rate at which cell metabolism proceeds by measuring the rate of ATP utilization. A typical mammalian cell "turns over" (i.e., hydrolyzes and restores by phosphorylation) its entire ATP pool once every 1 or 2 minutes. For each cell, this turnover represents the utilization of roughly 10^7 molecules of ATP per second (or, for the human body, about 1 gram of ATP every minute).

The rates of reactions in cells are rapid because enzyme catalysis is so effective. Many important enzymes have become so efficient that there is no possibility of further useful improvement. The factor that limits the reaction rate is no longer the enzyme's intrinsic speed of action; rather, it is the frequency with which the enzyme collides with its substrate. Such a reaction is said to be *diffusion-limited* (see Panel 3–3, p. 162–163).

If an enzyme-catalyzed reaction is diffusion-limited, its rate depends on the concentration of both the enzyme and its substrate. If a sequence of reactions is to occur extremely rapidly, each metabolic intermediate and enzyme involved must be present in high concentration. However, given the enormous number of different reactions performed by a cell, there are limits to the concentrations that can be achieved. In fact, most metabolites are present in micromolar (10^{-6} M) concentrations, and most enzyme concentrations are much lower. How is it possible, therefore, to maintain very fast metabolic rates?

The answer lies in the spatial organization of cell components. The cell can increase reaction rates without raising substrate concentrations by bringing the various enzymes involved in a reaction sequence together to form a large protein assembly known as a *multienzyme complex* (**Figure 3–55**). Because this allows the product of enzyme A to be passed directly to enzyme B, and so on, diffusion rates need not be limiting, even when the concentrations of the substrates in the cell as a whole are very low. It is perhaps not surprising, therefore, that such enzyme complexes are very common, and they are involved in nearly all aspects of metabolism—including the central genetic processes of DNA, RNA, and protein synthesis. In fact, few enzymes in eucaryotic cells diffuse freely in solution; instead, most seem to have evolved binding sites that concentrate them with other proteins of related function in particular regions of the cell, thereby increasing the rate and efficiency of the reactions that they catalyze.

Eucaryotic cells have yet another way of increasing the rate of metabolic reactions: using their intracellular membrane systems. These membranes can segregate particular substrates and the enzymes that act on them into the same membrane-enclosed compartment, such as the endoplasmic reticulum or the cell nucleus. If, for example, a compartment occupies a total of 10% of the volume of the cell, the concentration of reactants in that compartment may be increased by 10 times compared with a cell with the same number of enzymes and substrate molecules, but no compartmentalization. Reactions limited by the speed of diffusion can thereby be speeded up by a factor of 10.

The Cell Regulates the Catalytic Activities of its Enzymes

A living cell contains thousands of enzymes, many of which operate at the same time and in the same small volume of the cytosol. By their catalytic action, these enzymes generate a complex web of metabolic pathways, each composed of chains of chemical reactions in which the product of one enzyme becomes the substrate of the next. In this maze of pathways, there are many branch points (nodes) where different enzymes compete for the same substrate. The system is so complex (see Figure 2–88) that elaborate controls are required to regulate when and how rapidly each reaction occurs.

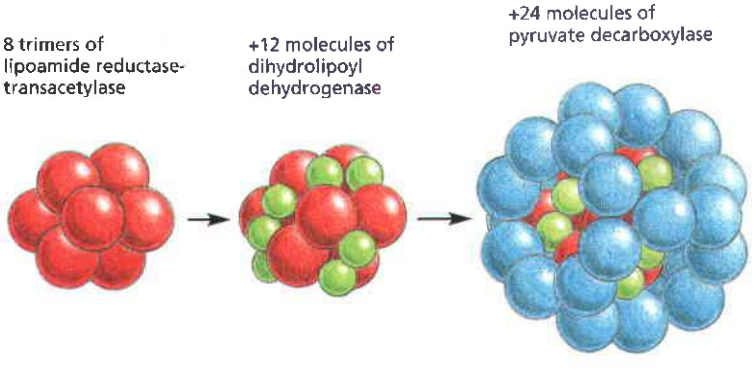


Figure 3–55 The structure of pyruvate dehydrogenase. This enzyme complex catalyzes the conversion of pyruvate to acetyl CoA, as part of the pathway that oxidizes sugars to CO_2 and H_2O (see Figure 2–79). It is an example of a large multienzyme complex in which reaction intermediates are passed directly from one enzyme to another.

Regulation occurs at many levels. At one level, the cell controls how many molecules of each enzyme it makes by regulating the expression of the gene that encodes that enzyme (discussed in Chapter 7). The cell also controls enzymatic activities by confining sets of enzymes to particular subcellular compartments, enclosed by distinct membranes (discussed in Chapters 12 and 14). As will be discussed later in this chapter, enzymes are frequently covalently modified to control their activity. The rate of protein destruction by targeted proteolysis represents yet another important regulatory mechanism (see p. 395). But the most general process that adjusts reaction rates operates through a direct, reversible change in the activity of an enzyme in response to the specific small molecules that it encounters.

The most common type of control occurs when a molecule other than one of the substrates binds to an enzyme at a special regulatory site outside the active site, thereby altering the rate at which the enzyme converts its substrates to products. For example, in **feedback inhibition** a product produced late in a reaction pathway inhibits an enzyme that acts earlier in the pathway. Thus, whenever large quantities of the final product begin to accumulate, this product binds to the enzyme and slows down its catalytic action, thereby limiting the further entry of substrates into that reaction pathway (**Figure 3–56**). Where pathways branch or intersect, there are usually multiple points of control by different final products, each of which works to regulate its own synthesis (**Figure 3–57**). Feedback inhibition can work almost instantaneously, and it is rapidly reversed when the level of the product falls.

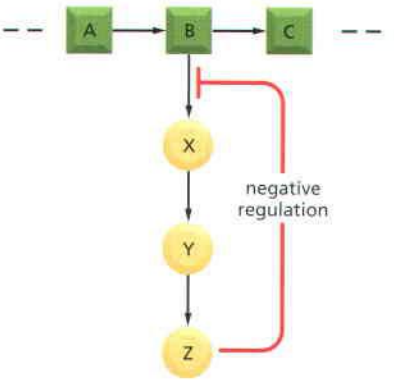


Figure 3–56 Feedback inhibition of a single biosynthetic pathway. The end-product Z inhibits the first enzyme that is unique to its synthesis and thereby controls its own level in the cell. This is an example of negative regulation.

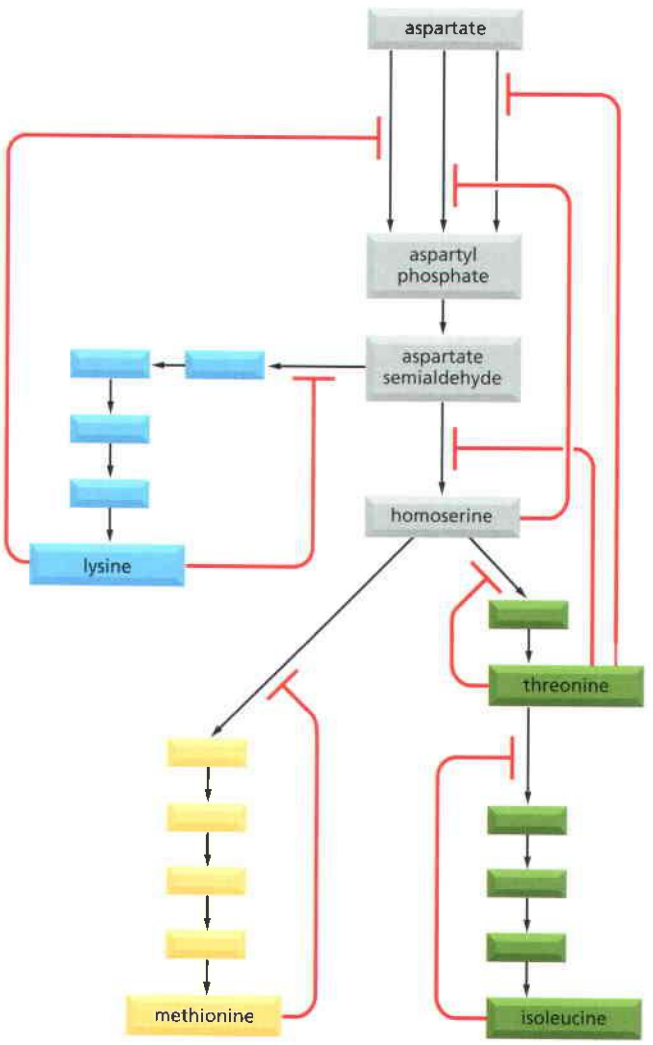


Figure 3–57 Multiple feedback inhibition. In this example, which shows the biosynthetic pathways for four different amino acids in bacteria, the *red arrows* indicate positions at which products feed back to inhibit enzymes. Each amino acid controls the first enzyme specific to its own synthesis, thereby controlling its own levels and avoiding a wasteful, or even dangerous, buildup of intermediates. The products can also separately inhibit the initial set of reactions common to all the syntheses; in this case, three different enzymes catalyze the initial reaction, each inhibited by a different product.

Feedback inhibition is *negative regulation:* it prevents an enzyme from acting. Enzymes can also be subject to *positive regulation,* in which a regulatory molecule stimulates the enzyme's activity rather than shutting the enzyme down. Positive regulation occurs when a product in one branch of the metabolic network stimulates the activity of an enzyme in another pathway. As one example, the accumulation of ADP activates several enzymes involved in the oxidation of sugar molecules, thereby stimulating the cell to convert more ADP to ATP.

Allosteric Enzymes Have Two or More Binding Sites That Interact

A striking feature of both positive and negative feedback regulation is that the regulatory molecule often has a shape totally different from the shape of the substrate of the enzyme. This is why the effect on a protein is termed *allostery* (from the Greek words *allos,* meaning "other," and *stereos,* meaning "solid" or "three-dimensional"). As biologists learned more about feedback regulation, they recognized that the enzymes involved must have at least two different binding sites on their surface—an **active site** that recognizes the substrates, and a **regulatory site** that recognizes a regulatory molecule. These two sites must somehow communicate so that the catalytic events at the active site can be influenced by the binding of the regulatory molecule at its separate site on the protein's surface.

The interaction between separated sites on a protein molecule is now known to depend on a *conformational change* in the protein: binding at one of the sites causes a shift from one folded shape to a slightly different folded shape. During feedback inhibition, for example, the binding of an inhibitor at one site on the protein causes the protein to shift to a conformation that incapacitates its active site, located elsewhere in the protein.

It is thought that most protein molecules are allosteric. They can adopt two or more slightly different conformations, and a shift from one to another caused by the binding of a ligand can alter their activity. This is true not only for enzymes but also for many other proteins, including receptors, structural proteins, and motor proteins. In all instances of allosteric regulation, each conformation of the protein has somewhat different surface contours, and the protein's binding sites for ligands are altered when the protein changes shape. Moreover as we discuss next, each ligand will stabilize the conformation that it binds to most strongly, and thus—at high enough concentrations—will tend to "switch" the protein toward the conformation that the ligand prefers.

Two Ligands Whose Binding Sites Are Coupled Must Reciprocally Affect Each Other's Binding

The effects of ligand binding on a protein follow from a fundamental chemical principle known as **linkage**. Suppose, for example, that a protein that binds glucose also binds another molecule, X, at a distant site on the protein's surface. If the binding site for X changes shape as part of the conformational change induced by glucose binding, the binding sites for X and for glucose are said to be *coupled.* Whenever two ligands prefer to bind to the *same* conformation of an allosteric protein, it follows from basic thermodynamic principles that each ligand must increase the affinity of the protein for the other. Thus, if the shift of the protein in **Figure 3–58** to the closed conformation that binds glucose best also causes the binding site for X to fit X better, then the protein will bind glucose more tightly when X is present than when X is absent.

Conversely, linkage operates in a negative way if two ligands prefer to bind to *different* conformations of the same protein. In this case, the binding of the first ligand discourages the binding of the second ligand. Thus, if a shape change caused by glucose binding decreases the affinity of a protein for molecule X, the binding of X must also decrease the protein's affinity for glucose (**Figure 3–59**). The linkage relationship is quantitatively reciprocal, so that, for example, if glucose has a very large effect on the binding of X, X has a very large effect on the binding of glucose.

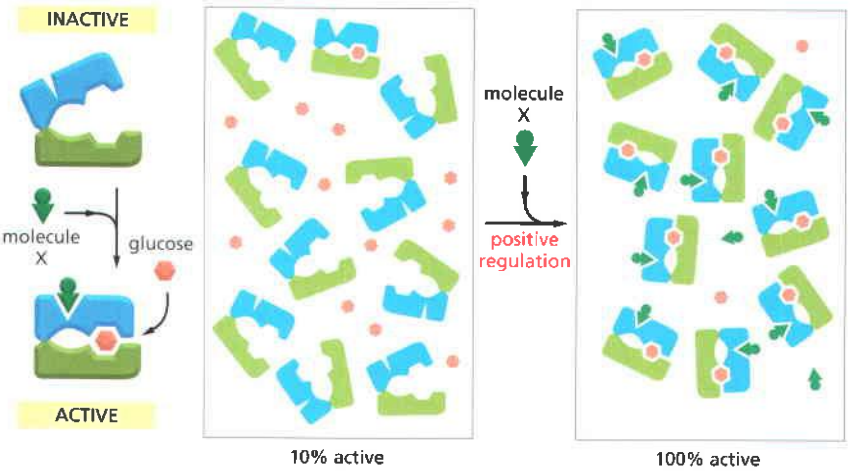


Figure 3–58 Positive regulation caused by conformational coupling between two distant binding sites. In this example, both glucose and molecule X bind best to the *closed* conformation of a protein with two domains. Because both glucose and molecule X drive the protein toward its closed conformation, each ligand helps the other to bind. Glucose and molecule X are therefore said to bind *cooperatively* to the protein.

The relationships shown in Figures 3–58 and 3–59 apply to all proteins, and they underlie all of cell biology. They seem so obvious in retrospect that we now take it for granted. But the discovery of linkage in studies of a few enzymes in the 1950s, followed by an extensive analysis of allosteric mechanisms in proteins in the early 1960s, had a revolutionary effect on our understanding of biology. Since molecule X in these examples binds at a site on the enzyme that is distinct from the site where catalysis occurs, it need have no chemical relationship to glucose or to any other ligand that binds at the active site. Moreover, as we have just seen, for enzymes that are regulated in this way, molecule X can either turn the enzyme on (positive regulation) or turn it off (negative regulation). By such a mechanism, **allosteric proteins** serve as general switches that, in principle, allow one molecule in a cell to affect the fate of any other.

Symmetric Protein Assemblies Produce Cooperative Allosteric Transitions

A single-subunit enzyme that is regulated by negative feedback can at most decrease from 90% to about 10% activity in response to a 100-fold increase in the concentration of an inhibitory ligand that it binds (**Figure 3–60**, *red line*). Responses of this type are apparently not sharp enough for optimal cell regulation, and most enzymes that are turned on or off by ligand binding consist of symmetric assemblies of identical subunits. With this arrangement, the binding of a molecule of ligand to a single site on one subunit can promote an allosteric change in the entire assembly that helps the neighboring subunits bind the same ligand. As a result, a *cooperative allosteric transition* occurs (Figure 3–60, *blue line*), allowing a relatively small change in ligand concentration in the cell to switch the whole assembly from an almost fully active to an almost fully inactive conformation (or vice versa).

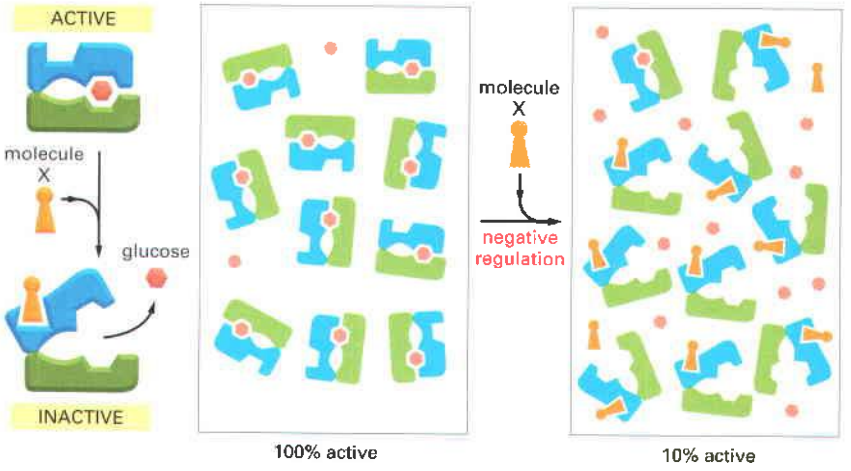


Figure 3–59 Negative regulation caused by conformational coupling between two distant binding sites. The scheme here resembles that in the previous figure, but here molecule X prefers the *open* conformation, while glucose prefers the *closed* conformation. Because glucose and molecule X drive the protein toward opposite conformations (closed and open, respectively), the presence of either ligand interferes with the binding of the other.

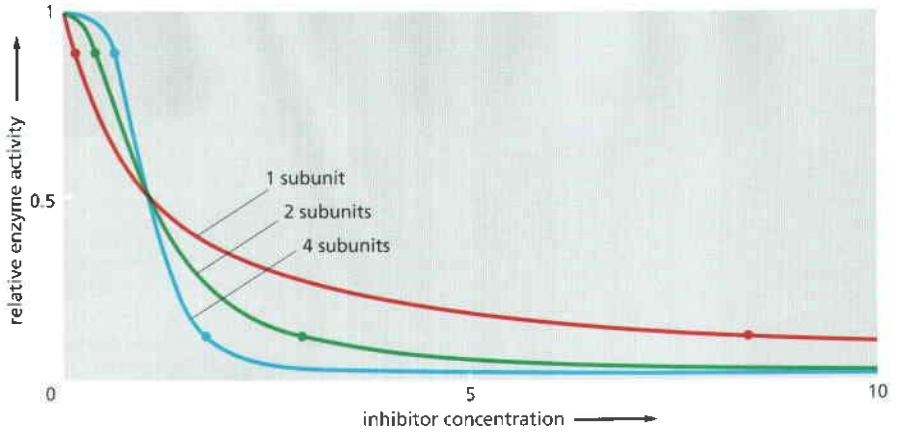


Figure 3–60 Enzyme activity versus the concentration of inhibitory ligand for single-subunit and multisubunit allosteric enzymes. For an enzyme with a single subunit *(red line)*, a drop from 90% enzyme activity to 10% activity (indicated by the two dots on the curve) requires a 100-fold increase in the concentration of inhibitor. The enzyme activity is calculated from the simple equilibrium relationship $K = [IP]/[I][P]$, where P is active protein, I is inhibitor, and IP is the inactive protein bound to inhibitor. An identical curve applies to any simple binding interaction between two molecules, A and B. In contrast, a multisubunit allosteric enzyme can respond in a switchlike manner to a change in ligand concentration: the steep response is caused by a cooperative binding of the ligand molecules, as explained in Figure 3–61. Here, the *green line* represents the idealized result expected for the cooperative binding of two inhibitory ligand molecules to an allosteric enzyme with two subunits, and the *blue line* shows the idealized response of an enzyme with four subunits. As indicated by the two dots on each of these curves, the more complex enzymes drop from 90% to 10% activity over a much narrower range of inhibitor concentration than does the enzyme composed of a single subunit.

The principles involved in a cooperative "all-or-none" transition are the same for all proteins, whether or not they are enzymes. But they are perhaps easiest to visualize for an enzyme that forms a symmetric dimer. In the example shown in **Figure 3–61**, the first molecule of an inhibitory ligand binds with great difficulty since its binding disrupts an energetically favorable interaction between the two identical monomers in the dimer. A second molecule of inhibitory ligand now binds more easily, however, because its binding restores the energetically favorable monomer–monomer contacts of a symmetric dimer (this also completely inactivates the enzyme).

As an alternative to this *induced fit* model for a cooperative allosteric transition, we can view such a symmetrical enzyme as having only two possible conformations, corresponding to the "enzyme on" and "enzyme off" structures in Figure 3–61. In this view, ligand binding perturbs an all-or-none equilibrium between these two states, thereby changing the proportion of active molecules. Both models represent true and useful concepts; it is the second model that we shall describe next.

The Allosteric Transition in Aspartate Transcarbamoylase Is Understood in Atomic Detail

One enzyme used in the early studies of allosteric regulation was aspartate transcarbamoylase from *E. coli*. It catalyzes the important reaction that begins the synthesis of the pyrimidine ring of C, U, and T nucleotides: carbamoyl phosphate + aspartate → *N*-carbamoylaspartate. One of the final products of this pathway, cytosine triphosphate (CTP), binds to the enzyme to turn it off whenever CTP is plentiful.

Aspartate transcarbamoylase is a large complex of six regulatory and six catalytic subunits. The catalytic subunits form two trimers, each arranged in the shape of an equilateral triangle; the two trimers face each other and are held

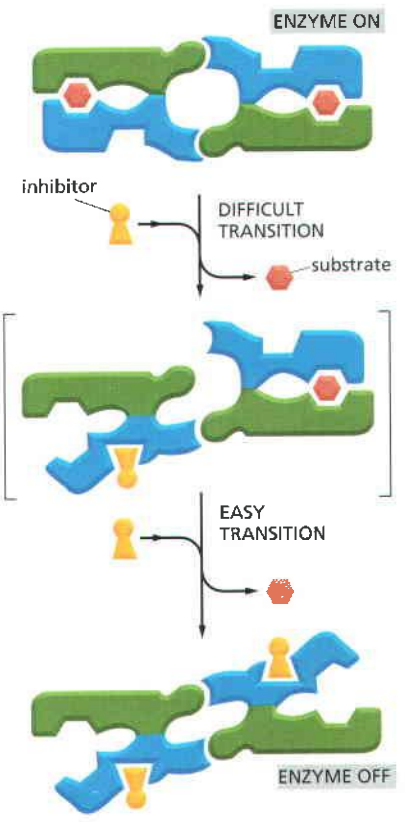


Figure 3–61 A cooperative allosteric transition in an enzyme composed of two identical subunits. This diagram illustrates how the conformation of one subunit can influence that of its neighbor. The binding of a single molecule of an inhibitory ligand *(yellow)* to one subunit of the enzyme occurs with difficulty because it changes the conformation of this subunit and thereby disrupts the symmetry of the enzyme. Once this conformational change has occurred, however, the energy gained by restoring the symmetric pairing interaction between the two subunits makes it especially easy for the second subunit to bind the inhibitory ligand and undergo the same conformational change. Because the binding of the first molecule of ligand increases the affinity with which the other subunit binds the same ligand, the response of the enzyme to changes in the concentration of the ligand is much steeper than the response of an enzyme with only one subunit (see Figure 3–60).

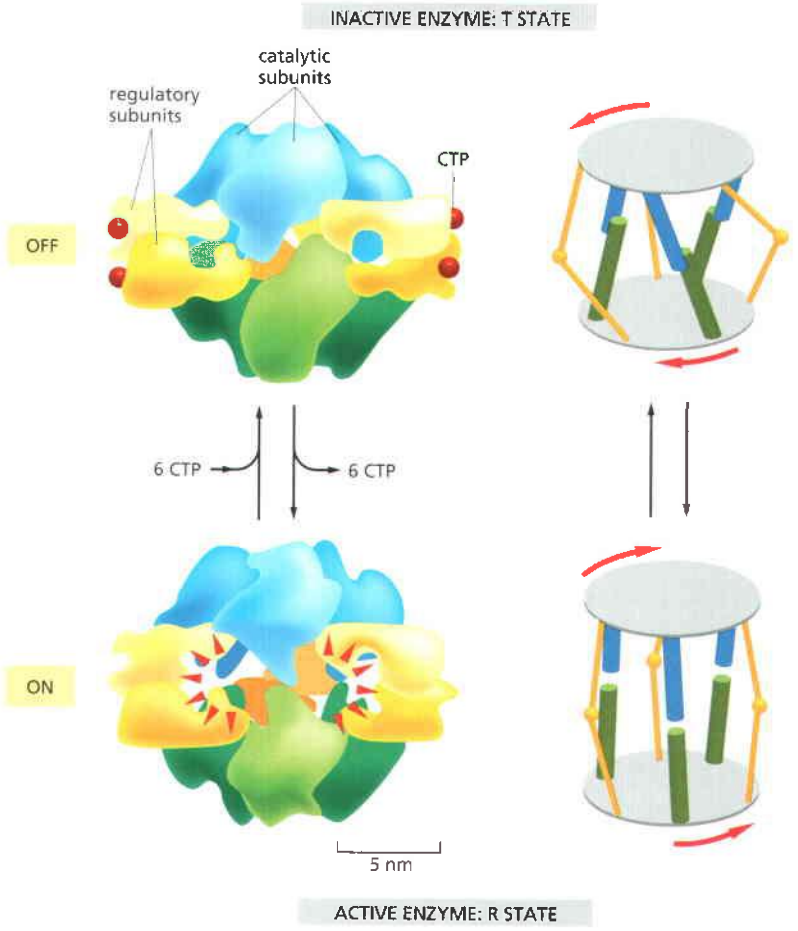


Figure 3–62 The transition between R and T states in the enzyme aspartate transcarbamoylase. <CTAA> The enzyme consists of a complex of six catalytic subunits and six regulatory subunits, and the structures of its inactive (T state) and active (R state) forms have been determined by x-ray crystallography. The enzyme is turned off by feedback inhibition when CTP concentrations rise. Each regulatory subunit can bind one molecule of CTP, which is one of the final products in the pathway. By means of this negative feedback regulation, the pathway is prevented from producing more CTP than the cell needs. (Based on K.L. Krause, K.W. Volz and W.N. Lipscomb, *Proc. Natl Acad. Sci. U.S.A.* 82:1643–1647, 1985. With permission from National Academy of Sciences.)

together by three regulatory dimers that form a bridge between them. The entire molecule is poised to undergo a concerted, all-or-none, allosteric transition between two conformations, designated as T (tense) and R (relaxed) states (**Figure 3–62**).

The binding of substrates (carbamoyl phosphate and aspartate) to the catalytic trimers drives aspartate transcarbamoylase into its catalytically active R state, from which the regulatory CTP molecules dissociate. By contrast, the binding of CTP to the regulatory dimers converts the enzyme to the inactive T state, from which the substrates dissociate. This tug-of-war between CTP and substrates is identical in principle to that described previously in Figure 3–59 for a simpler allosteric protein. But because the tug-of-war occurs in a symmetric molecule with multiple binding sites, the enzyme undergoes a cooperative allosteric transition that will turn it on suddenly as substrates accumulate (forming the R state) or shut it off rapidly when CTP accumulates (forming the T state).

A combination of biochemistry and x-ray crystallography has revealed many fascinating details of this allosteric transition. Each regulatory subunit has two domains, and the binding of CTP causes the two domains to move relative to each other, so that they function like a lever that rotates the two catalytic trimers and pulls them closer together into the T state (see Figure 3–62). When this occurs, hydrogen bonds form between opposing catalytic subunits. This helps widen the cleft that forms the active site within each catalytic subunit, thereby disrupting the binding sites for the substrates (**Figure 3–63**). Adding large amounts of substrate has the opposite effect, favoring the R state by binding in the cleft of each catalytic subunit and opposing the above conformational change. Conformations that are intermediate between R and T are unstable, so that the enzyme mostly clicks back and forth between its R and T forms, producing a mixture of these two species in proportions that depend on the relative concentrations of CTP and substrates.

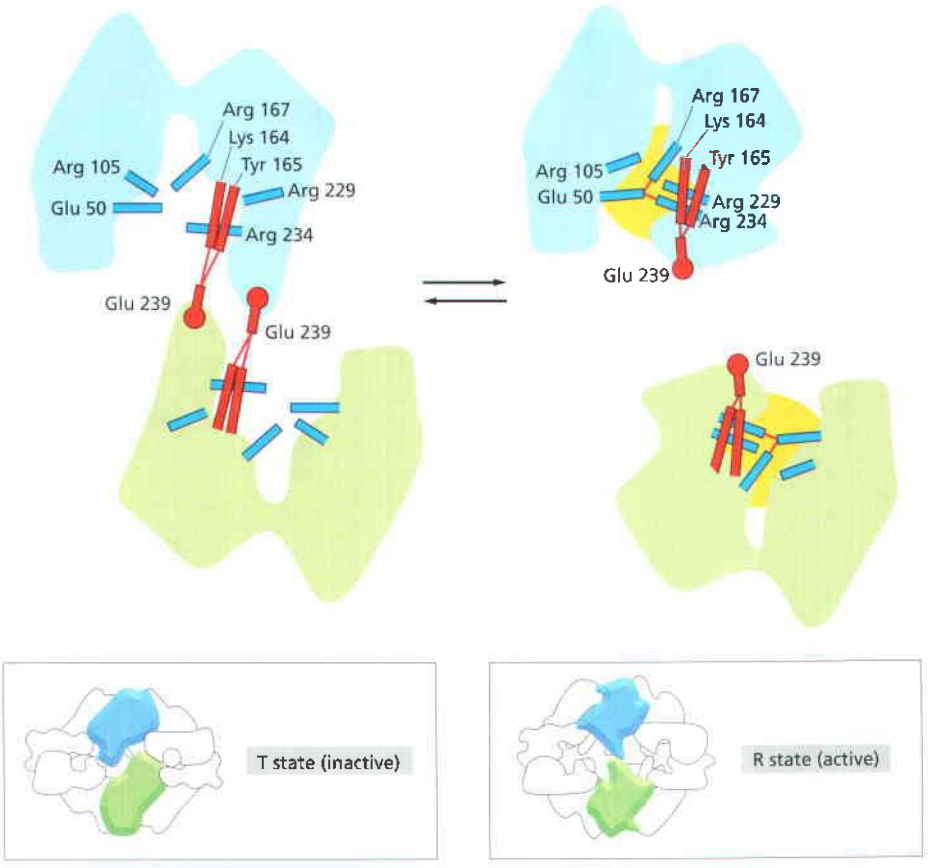


Figure 3–63 Part of the on–off switch in the catalytic subunits of aspartate transcarbamoylase. Changes in the indicated hydrogen-bonding interactions are partly responsible for switching this enzyme's active site between active *(yellow)* and inactive conformations. Hydrogen bonds are indicated by *thin red lines*. The amino acids involved in the subunit–subunit interaction in the T state are shown in *red*, while those that form the active site of the enzyme in the R state are shown in *blue*. The large drawings show the catalytic site in the interior of the enzyme; the boxed sketches show the same subunits viewed from the enzyme's external surface. (Adapted from E.R. Kantrowitz and W.N. Lipscomb, *Trends Biochem. Sci.* 15:53–59, 1990. With permission from Elsevier.)

Many Changes in Proteins Are Driven by Protein Phosphorylation

Proteins are regulated by more than the reversible binding of other molecules. A second method that eucaryotic cells use to regulate a protein's function is the covalent addition of a smaller molecule to one or more of its amino acid side chains. The most common such regulatory modification in higher eucaryotes is the addition of a phosphate group. We shall therefore use protein phosphorylation to illustrate some of the general principles involved in the control of protein function through the modification of amino acid side chains.

A phosphorylation event can affect the protein that is modified in two important ways. First, because each phosphate group carries two negative charges, the enzyme-catalyzed addition of a phosphate group to a protein can cause a major conformational change in the protein by, for example, attracting a cluster of positively charged amino acid side chains. This can, in turn, affect the binding of ligands elsewhere on the protein surface, dramatically changing the protein's activity. When a second enzyme removes the phosphate group, the protein returns to its original conformation and restores its initial activity.

Second, an attached phosphate group can form part of a structure that the binding sites of other proteins recognize. As previously discussed, certain protein domains, sometimes referred to as modules, appear very frequently as parts of larger proteins. One such module is the SH2 domain, described earlier, which binds to a short peptide sequence containing a phosphorylated tyrosine side chain (see Figure 3–39B). More than ten other common domains provide binding sites for attaching their protein to phosphorylated peptides in other protein molecules, each recognizing a phosphorylated amino acid side chain in a different protein context. As a result, protein phosphorylation and dephosphorylation very often drive the regulated assembly and disassembly of protein complexes (see Figure 15–22).

Reversible protein phosphorylation controls the activity, structure, and cellular localization of both enzymes and many other types of proteins in

eucaryotic cells. In fact, this regulation is so extensive that more than one-third of the 10,000 or so proteins in a typical mammalian cell are thought to be phosphorylated at any given time—many with more than one phosphate. As might be expected, the addition and removal of phosphate groups from specific proteins often occur in response to signals that specify some change in a cell's state. For example, the complicated series of events that takes place as a eucaryotic cell divides is largely timed in this way (discussed in Chapter 17), and many of the signals mediating cell–cell interactions are relayed from the plasma membrane to the nucleus by a cascade of protein phosphorylation events (discussed in Chapter 15).

A Eucaryotic Cell Contains a Large Collection of Protein Kinases and Protein Phosphatases

Protein phosphorylation involves the enzyme-catalyzed transfer of the terminal phosphate group of an ATP molecule to the hydroxyl group on a serine, threonine, or tyrosine side chain of the protein (**Figure 3–64**). A **protein kinase** catalyzes this reaction, and the reaction is essentially unidirectional because of the large amount of free energy released when the phosphate–phosphate bond in ATP is broken to produce ADP (discussed in Chapter 2). A **protein phosphatase** catalyzes the reverse reaction of phosphate removal, or *dephosphorylation*. Cells contain hundreds of different protein kinases, each responsible for phosphorylating a different protein or set of proteins. There are also many different protein phosphatases; some are highly specific and remove phosphate groups from only one or a few proteins, whereas others act on a broad range of proteins and are targeted to specific substrates by regulatory subunits. The state of phosphorylation of a protein at any moment, and thus its activity, depends on the relative activities of the protein kinases and phosphatases that modify it.

The protein kinases that phosphorylate proteins in eucaryotic cells belong to a very large family of enzymes, which share a catalytic (kinase) sequence of about 290 amino acids. The various family members contain different amino acid sequences on either end of the kinase sequence (for example, see Figure 3–10), and often have short amino acid sequences inserted into loops within it (*red arrowheads* in **Figure 3–65**). Some of these additional amino acid sequences enable each kinase to recognize the specific set of proteins it phosphorylates, or to bind to structures that localize it in specific regions of the cell. Other parts of the protein regulate the activity of each kinase, so it can be turned on and off in response to different specific signals, as described below.

By comparing the number of amino acid sequence differences between the various members of a protein family, we can construct an "evolutionary tree" that is thought to reflect the pattern of gene duplication and divergence that gave rise to the family. **Figure 3–66** shows an evolutionary tree of protein kinases. Kinases with related functions are often located on nearby branches of the tree: the protein kinases involved in cell signaling that phosphorylate tyrosine side chains, for example, are all clustered in the top left corner of the tree. The other kinases shown phosphorylate either a serine or a threonine side chain, and many are organized into clusters that seem to reflect their function—in transmembrane signal transduction, intracellular signal amplification, cell-cycle control, and so on.

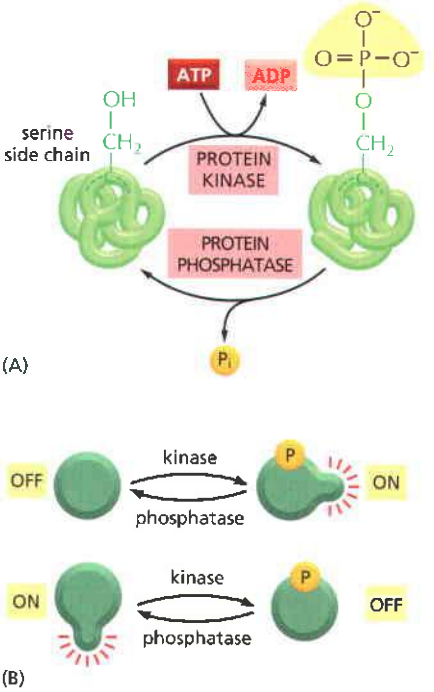


Figure 3–64 Protein phosphorylation. Many thousands of proteins in a typical eucaryotic cell are modified by the covalent addition of a phosphate group. (A) The general reaction, shown here, transfers a phosphate group from ATP to an amino acid side chain of the target protein by a protein kinase. Removal of the phosphate group is catalyzed by a second enzyme, a protein phosphatase. In this example, the phosphate is added to a serine side chain; in other cases, the phosphate is instead linked to the –OH group of a threonine or a tyrosine in the protein. (B) The phosphorylation of a protein by a protein kinase can either increase or decrease the protein's activity, depending on the site of phosphorylation and the structure of the protein.

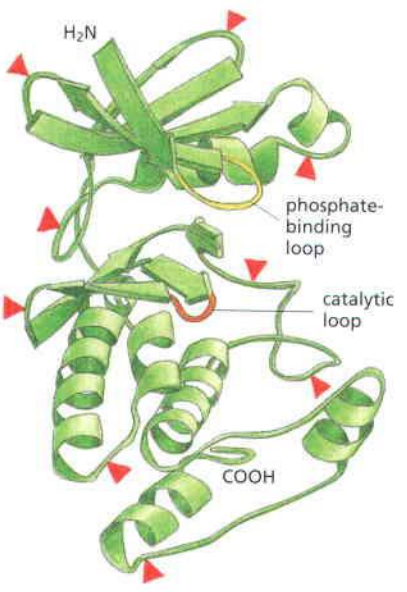


Figure 3–65 The three-dimensional structure of a protein kinase. Superimposed on this structure are *red arrowheads* to indicate sites where insertions of 5–100 amino acids are found in some members of the protein kinase family. These insertions are located in loops on the surface of the enzyme where other ligands interact with the protein. Thus, they distinguish different kinases and confer on them distinctive interactions with other proteins. The ATP (which donates a phosphate group) and the peptide to be phosphorylated are held in the active site, which extends between the phosphate-binding loop *(yellow)* and the catalytic loop *(orange)*. See also Figure 3–10. (Adapted from D.R. Knighton et al., *Science* 253:407–414, 1991. With permission from AAAS.)

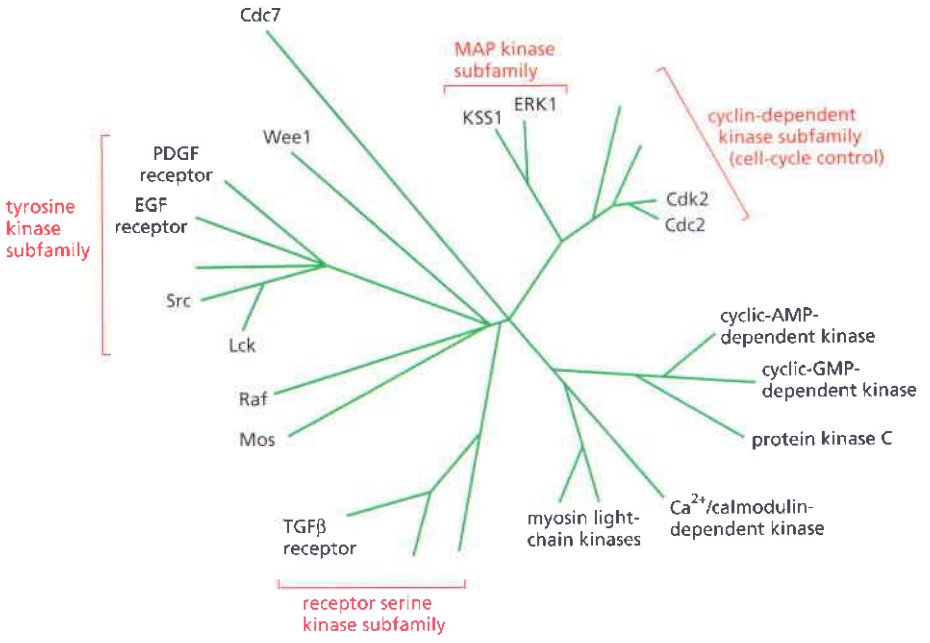


Figure 3–66 An evolutionary tree of selected protein kinases. Although a higher eucaryotic cell contains hundreds of such enzymes, and the human genome codes for more than 500, only some of those discussed in this book are shown.

As a result of the combined activities of protein kinases and protein phosphatases, the phosphate groups on proteins are continually turning over—being added and then rapidly removed. Such phosphorylation cycles may seem wasteful, but they are important in allowing the phosphorylated proteins to switch rapidly from one state to another: the more rapid the cycle, the faster a population of protein molecules can change its state of phosphorylation in response to a sudden change in the phosphorylation rate (see Figure 15–11). The energy required to drive this phosphorylation cycle is derived from the free energy of ATP hydrolysis, one molecule of which is consumed for each phosphorylation event.

The Regulation of Cdk and Src Protein Kinases Shows How a Protein Can Function as a Microchip

The hundreds of different protein kinases in a eucaryotic cell are organized into complex networks of signaling pathways that help to coordinate the cell's activities, drive the cell cycle, and relay signals into the cell from the cell's environment. Many of the extracellular signals involved need to be both integrated and amplified by the cell. Individual protein kinases (and other signaling proteins) serve as input–output devices, or "microchips," in the integration process. An important part of the input to these signal processing proteins comes from the control that is exerted by phosphates added and removed from them by protein kinases and protein phosphatases, respectively.

In general, specific sets of phosphate groups serve to activate the protein, while other sets can inactivate it. A cyclin-dependent protein kinase (Cdk) provides a good example. Kinases in this class phosphorylate serines and threonines, and they are central components of the cell-cycle control system in eucaryotic cells, as discussed in detail in Chapter 17. In a vertebrate cell, individual Cdk proteins turn on and off in succession, as a cell proceeds through the different phases of its division cycle. When a particular kinase is on, it influences various aspects of cell behavior through effects on the proteins it phosphorylates.

A Cdk protein becomes active as a serine/threonine protein kinase only when it is bound to a second protein called a *cyclin*. But, as **Figure 3–67** shows, the binding of cyclin is only one of three distinct "inputs" required to activate the Cdk. In addition to cyclin binding, a phosphate must be added to a specific threonine side chain, and a phosphate elsewhere in the protein (covalently bound to a specific tyrosine side chain) must be removed. Cdk thus monitors a specific set

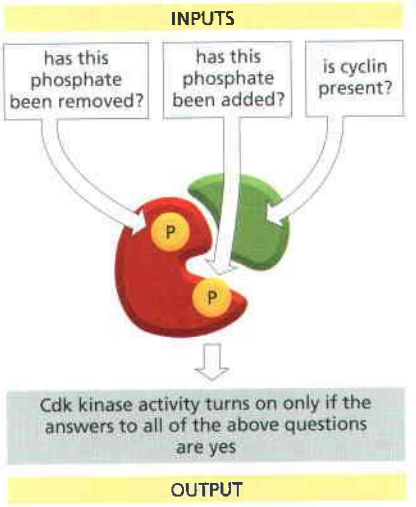


Figure 3–67 How a Cdk protein acts as an integrating device. <TAGA> The function of these central regulators of the cell cycle is discussed in Chapter 17.



Figure 3–68 The domain structure of the Src family of protein kinases, mapped along the amino acid sequence. For the three-dimensional structure of Src, see Figure 3–10.

of cell components—a cyclin, a protein kinase, and a protein phosphatase—and it acts as an input–output device that turns on if, and only if, each of these components has attained its appropriate activity state. Some cyclins rise and fall in concentration in step with the cell cycle, increasing gradually in amount until they are suddenly destroyed at a particular point in the cycle. The sudden destruction of a cyclin (by targeted proteolysis) immediately shuts off its partner Cdk enzyme, and this triggers a specific step in the cell cycle.

The Src family of protein kinases (see Figure 3–10) exhibits a similar type of microchip behavior. The *Src protein* (pronounced "sarc" and named for the type of tumor, a sarcoma, that its deregulation can cause) was the first tyrosine kinase to be discovered. It is now known to be part of a subfamily of nine very similar protein kinases, which are found only in multicellular animals. As indicated by the evolutionary tree in Figure 3–66, sequence comparisons suggest that tyrosine kinases as a group were a relatively late innovation that branched off from the serine/threonine kinases, with the Src subfamily being only one subgroup of the tyrosine kinases created in this way.

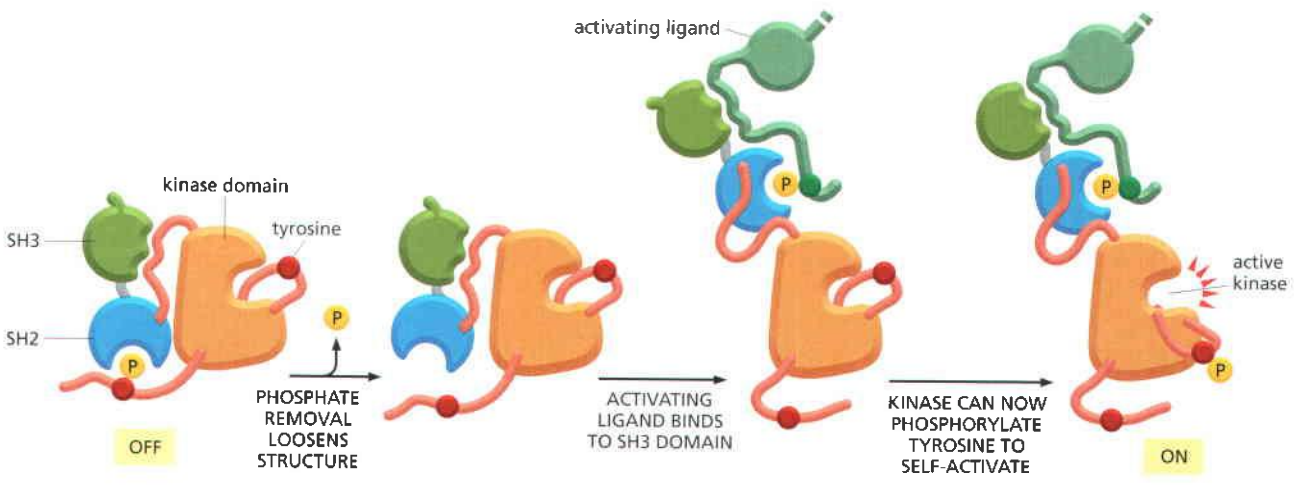
The Src protein and its relatives contain a short N-terminal region that becomes covalently linked to a strongly hydrophobic fatty acid, which holds the kinase at the cytoplasmic face of the plasma membrane. Next come two peptide-binding modules, a Src homology 3 (SH3) domain and a SH2 domain, followed by the kinase catalytic domain (**Figure 3–68**). These kinases normally exist in an inactive conformation, in which a phosphorylated tyrosine near the C-terminus is bound to the SH2 domain, and the SH3 domain is bound to an internal peptide in a way that distorts the active site of the enzyme and helps to render it inactive.

Turning the kinase on involves at least two specific inputs: removal of the C-terminal phosphate and the binding of the SH3 domain by a specific activating protein (**Figure 3–69**). Like the activation of the Cdk protein, the activation of the Src kinase signals the completion of a particular set of separate upstream events (**Figure 3–70**). Thus, both the Cdk and Src families of proteins serve as specific signal integrators, helping to generate the complex web of information-processing events that enable the cell to compute logical responses to a complex set of conditions.

Proteins That Bind and Hydrolyze GTP Are Ubiquitous Cellular Regulators

We have described how the addition or removal of phosphate groups on a protein can be used by a cell to control the protein's activity. In the examples discussed so

Figure 3–69 The activation of a Src-type protein kinase by two sequential events. (Adapted from S.C. Harrison et al., *Cell* 112:737–740, 2003. With permission from Elsevier.)



far, the phosphate is transferred from an ATP molecule to an amino acid side chain of the protein in a reaction catalyzed by a specific protein kinase. Eucaryotic cells also have another way to control protein activity by phosphate addition and removal. In this case, the phosphate is not attached directly to the protein; instead, it is a part of the guanine nucleotide GTP, which binds very tightly to the protein. In general, proteins regulated in this way are in their active conformations with GTP bound. The loss of a phosphate group occurs when the bound GTP is hydrolyzed to GDP in a reaction catalyzed by the protein itself, and in its GDP-bound state the protein is inactive. In this way, GTP-binding proteins act as on–off switches whose activity is determined by the presence or absence of an additional phosphate on a bound GDP molecule (**Figure 3–71**).

GTP-binding proteins (also called **GTPases** because of the GTP hydrolysis they catalyze) comprise a large family of proteins that all contain variations on the same GTP-binding globular domain. When the tightly bound GTP is hydrolyzed to GDP, this domain undergoes a conformational change that inactivates it. The three-dimensional structure of a prototypical member of this family, the monomeric GTPase called Ras, is shown in **Figure 3–72**.

The *Ras protein* has an important role in cell signaling (discussed in Chapter 15). In its GTP-bound form, it is active and stimulates a cascade of protein phosphorylations in the cell. Most of the time, however, the protein is in its inactive, GDP-bound form. It becomes active when it exchanges its GDP for a GTP molecule in response to extracellular signals, such as growth factors, that bind to receptors in the plasma membrane (see Figure 15–58).

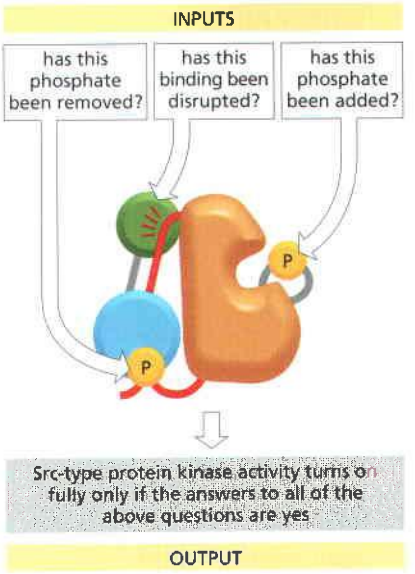


Figure 3–70 How a Src-type protein kinase acts as an integrating device. The disruption of the SH3 domain interaction *(green)* involves replacing its binding to the indicated *red* linker region with a tighter interaction with an activating ligand, as illustrated in Figure 3–69.

Regulatory Proteins Control the Activity of GTP-Binding Proteins by Determining Whether GTP or GDP Is Bound

GTP-binding proteins are controlled by regulatory proteins that determine whether GTP or GDP is bound, just as phosphorylated proteins are turned on and off by protein kinases and protein phosphatases. Thus, Ras is inactivated by a *GTPase-activating protein (GAP)*, which binds to the Ras protein and induces it to hydrolyze its bound GTP molecule to GDP—which remains tightly bound—and inorganic phosphate (P_i), which is rapidly released. The Ras protein stays in its inactive, GDP-bound conformation until it encounters a *guanine nucleotide exchange factor (GEF)*, which binds to GDP-Ras and causes it to release its GDP. Because the empty nucleotide-binding site is immediately filled by a GTP molecule (GTP is present in large excess over GDP in cells), the GEF activates Ras by *indirectly* adding back the phosphate removed by GTP hydrolysis. Thus, in a sense, the roles of GAP and GEF are analogous to those of a protein phosphatase and a protein kinase, respectively (**Figure 3–73**).

Large Protein Movements Can Be Generated From Small Ones

The Ras protein belongs to a large superfamily of *monomeric GTPases*, each of which consists of a single GTP-binding domain of about 200 amino acids. Over the course of evolution, this domain has also become joined to larger proteins with additional domains, creating a large family of GTP-binding proteins. Family members include the receptor-associated trimeric G proteins involved in cell signaling (discussed in Chapter 15), proteins regulating the traffic of vesicles between intracellular compartments (discussed in Chapter 13), and proteins that bind to transfer RNA and are required as assembly factors for protein

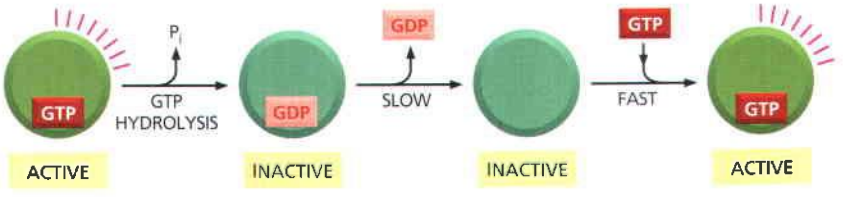


Figure 3–71 GTP-binding proteins as molecular switches. The activity of a GTP-binding protein (also called a GTPase) generally requires the presence of a tightly bound GTP molecule (switch "on"). Hydrolysis of this GTP molecule produces GDP and inorganic phosphate (P_i), and it causes the protein to convert to a different, usually inactive, conformation (switch "off"). As shown here, resetting the switch requires the tightly bound GDP to dissociate, a slow step that is greatly accelerated by specific signals; once the GDP has dissociated, a molecule of GTP is quickly rebound.

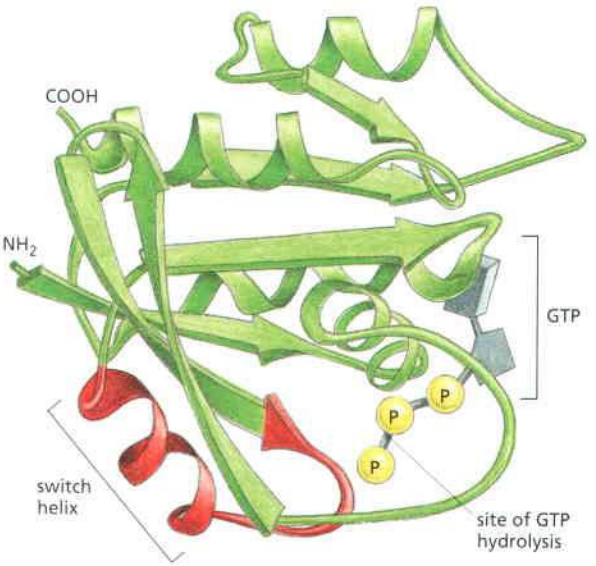


Figure 3–72 The structure of the Ras protein in its GTP-bound form. <GAAC> This monomeric GTPase illustrates the structure of a GTP-binding domain, which is present in a large family of GTP-binding proteins. The *red* regions change their conformation when the GTP molecule is hydrolyzed to GDP and inorganic phosphate by the protein; the GDP remains bound to the protein, while the inorganic phosphate is released. The special role of the "switch helix" in proteins related to Ras is explained next (see Figure 3–75).

synthesis on the ribosome (discussed in Chapter 6). In each case, an important biological activity is controlled by a change in the protein's conformation that is caused by GTP hydrolysis in a Ras-like domain.

The *EF-Tu protein* provides a good example of how this family of proteins works. EF-Tu is an abundant molecule that serves as an elongation factor (hence the EF) in protein synthesis, loading each aminoacyl tRNA molecule onto the ribosome. The tRNA molecule forms a tight complex with the GTP-bound form of EF-Tu (**Figure 3–74**). In this complex, the amino acid attached to the tRNA is improperly positioned for protein synthesis. The tRNA can transfer its amino acid only after the GTP bound to EF-Tu is hydrolyzed on the ribosome, allowing the EF-Tu to dissociate. Since the GTP hydrolysis is triggered by a proper fit of the tRNA to the mRNA molecule on the ribosome, the EF-Tu serves as a factor that discriminates between correct and incorrect mRNA–tRNA pairings (see Figure 6–67 for a further discussion of this function of EF-Tu).

By comparing the three-dimensional structure of EF-Tu in its GTP-bound and GDP-bound forms, we can see how the repositioning of the tRNA occurs. The dissociation of the inorganic phosphate group (P_i), which follows the reaction GTP → GDP + P_i, causes a shift of a few tenths of a nanometer at the GTP-binding site, just as it does in the Ras protein. This tiny movement, equivalent to

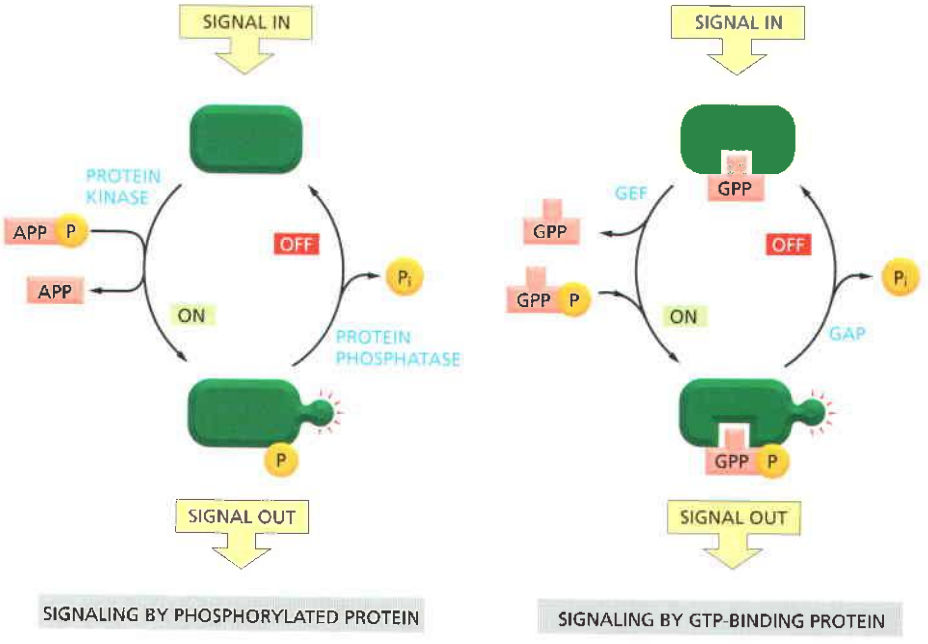


Figure 3–73 A comparison of the two major intracellular signaling mechanisms in eucaryotic cells. In both cases, a signaling protein is activated by the addition of a phosphate group and inactivated by the removal of this phosphate. To emphasize the similarities in the two pathways, ATP and GTP are drawn as APPP and GPPP, and ADP and GDP as APP and GPP, respectively. As shown in Figure 3–64, the addition of a phosphate to a protein can also be inhibitory.

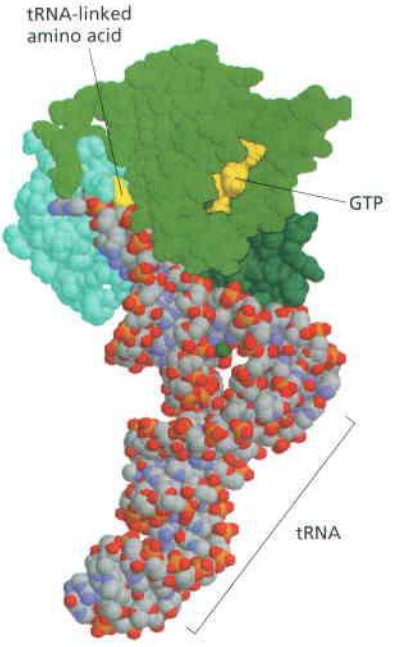


Figure 3–74 An aminoacyl tRNA molecule bound to EF-Tu. The three domains of the EF-Tu protein are colored differently, to match Figure 3–75. This is a bacterial protein; however, a very similar protein exists in eucaryotes, where it is called EF-1. (Coordinates determined by P. Nissen et al., *Science* 270:1464–1472, 1995. With permission from AAAS.)

a few times the diameter of a hydrogen atom, causes a conformational change to propagate along a crucial piece of α helix, called the *switch helix*, in the Ras-like domain of the protein. The switch helix seems to serve as a latch that adheres to a specific site in another domain of the molecule, holding the protein in a "shut" conformation. The conformational change triggered by GTP hydrolysis causes the switch helix to detach, allowing separate domains of the protein to swing apart, through a distance of about 4 nm. This releases the bound tRNA molecule, allowing its attached amino acid to be used (**Figure 3–75**).

Notice in this example how cells have exploited a simple chemical change that occurs on the surface of a small protein domain to create a movement 50 times larger. Dramatic shape changes of this type also cause the very large movements that occur in motor proteins, as we discuss next.

Motor Proteins Produce Large Movements in Cells

We have seen that conformational changes in proteins have a central role in enzyme regulation and cell signaling. We now discuss proteins whose major function is to move other molecules. These **motor proteins** generate the forces responsible for muscle contraction and the crawling and swimming of cells. Motor proteins also power smaller-scale intracellular movements: they help to move chromosomes to opposite ends of the cell during mitosis (discussed in Chapter 17), to move organelles along molecular tracks within the cell (discussed

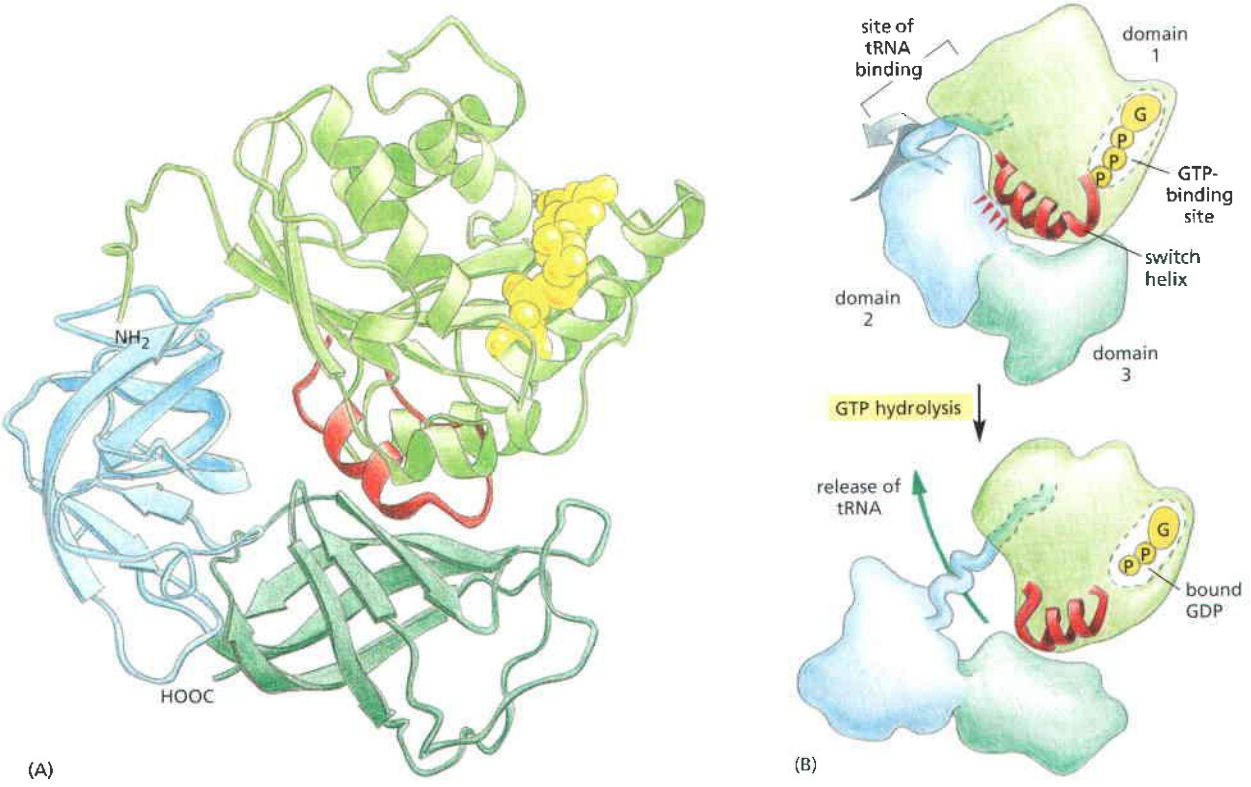


Figure 3–75 The large conformational change in EF-Tu caused by GTP hydrolysis. <GTAA> (A) The three-dimensional structure of EF-Tu with GTP bound. The domain at the top has a structure similar to the Ras protein, and its *red* α helix is the switch helix, which moves after GTP hydrolysis. (B) The change in the conformation of the switch helix in domain 1 causes domains 2 and 3 to rotate as a single unit by about 90° toward the viewer, which releases the tRNA that was shown bound to this structure in Figure 3–74. (A, adapted from H. Berchtold et al., *Nature* 365:126–132, 1993. With permission from Macmillan Publishers Ltd. B, courtesy of Mathias Sprinzl and Rolf Hilgenfeld.)

in Chapter 16), and to move enzymes along a DNA strand during the synthesis of a new DNA molecule (discussed in Chapter 5). All these fundamental processes depend on proteins with moving parts that operate as force-generating machines.

How do these machines work? In other words, how do cells use shape changes in proteins to generate directed movements? If, for example, a protein is required to walk along a narrow thread such as a DNA molecule, it can do this by undergoing a series of conformational changes, such as those shown in **Figure 3–76**. But with nothing to drive these changes in an orderly sequence, they are perfectly reversible, and the protein can only wander randomly back and forth along the thread. We can look at this situation in another way. Since the directional movement of a protein does work, the laws of thermodynamics (discussed in Chapter 2) demand that such movement use free energy from some other source (otherwise the protein could be used to make a perpetual motion machine). Therefore, without an input of energy, the protein molecule can only wander aimlessly.

How can the cell make such a series of conformational changes unidirectional? To force the entire cycle to proceed in one direction, it is enough to make any one of the changes in shape irreversible. Most proteins that are able to walk in one direction for long distances achieve this motion by coupling one of the conformational changes to the hydrolysis of an ATP molecule bound to the protein. The mechanism is similar to the one just discussed that drives allosteric protein shape changes by GTP hydrolysis. Because ATP (or GTP) hydrolysis releases a great deal of free energy, it is very unlikely that the nucleotide-binding protein will undergo the reverse shape change needed for moving backward—since this would require that it also reverse the ATP hydrolysis by adding a phosphate molecule to ADP to form ATP.

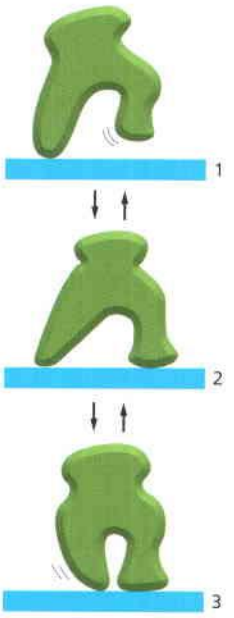


Figure 3–76 An allosteric "walking" protein. Although its three different conformations allow it to wander randomly back and forth while bound to a thread or a filament, the protein cannot move uniformly in a single direction.

In the model shown in **Figure 3–77**, ATP binding shifts a motor protein from conformation 1 to conformation 2. The bound ATP is then hydrolyzed to produce ADP and inorganic phosphate (P_i), causing a change from conformation 2 to conformation 3. Finally, the release of the bound ADP and P_i drives the protein back to conformation 1. Because the energy provided by ATP hydrolysis drives the transition 2 → 3, this series of conformational changes is effectively irreversible. Thus, the entire cycle goes in only one direction, causing the protein molecule to walk continuously to the right in this example.

Many motor proteins generate directional movement in this general way, including the muscle motor protein *myosin*, which walks along actin filaments to generate muscle contraction, and the *kinesin* proteins that walk along microtubules (both discussed in Chapter 16). These movements can be rapid: some of the motor proteins involved in DNA replication (the DNA helicases) propel themselves along a DNA strand at rates as high as 1000 nucleotides per second.

Membrane-Bound Transporters Harness Energy to Pump Molecules Through Membranes

We have thus far seen how allosteric proteins can act as microchips (Cdk and Src kinases), as assembly factors (EF-Tu), and as generators of mechanical force and motion (motor proteins). Allosteric proteins can also harness energy derived from ATP hydrolysis, ion gradients, or electron transport processes to pump specific ions or small molecules across a membrane. We consider one example here; others will be discussed in Chapter 11.

The ABC transporters constitute an important class of membrane-bound pump proteins. In humans at least 48 different genes encode them. These transporters mostly function to export hydrophobic molecules from the cytoplasm,

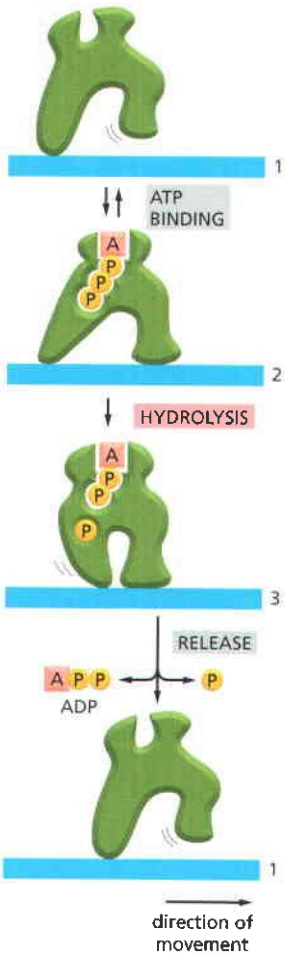


Figure 3–77 An allosteric motor protein. The transition between three different conformations includes a step driven by the hydrolysis of a bound ATP molecule, and this makes the entire cycle essentially irreversible. By repeated cycles, the protein therefore moves continuously to the right along the thread.

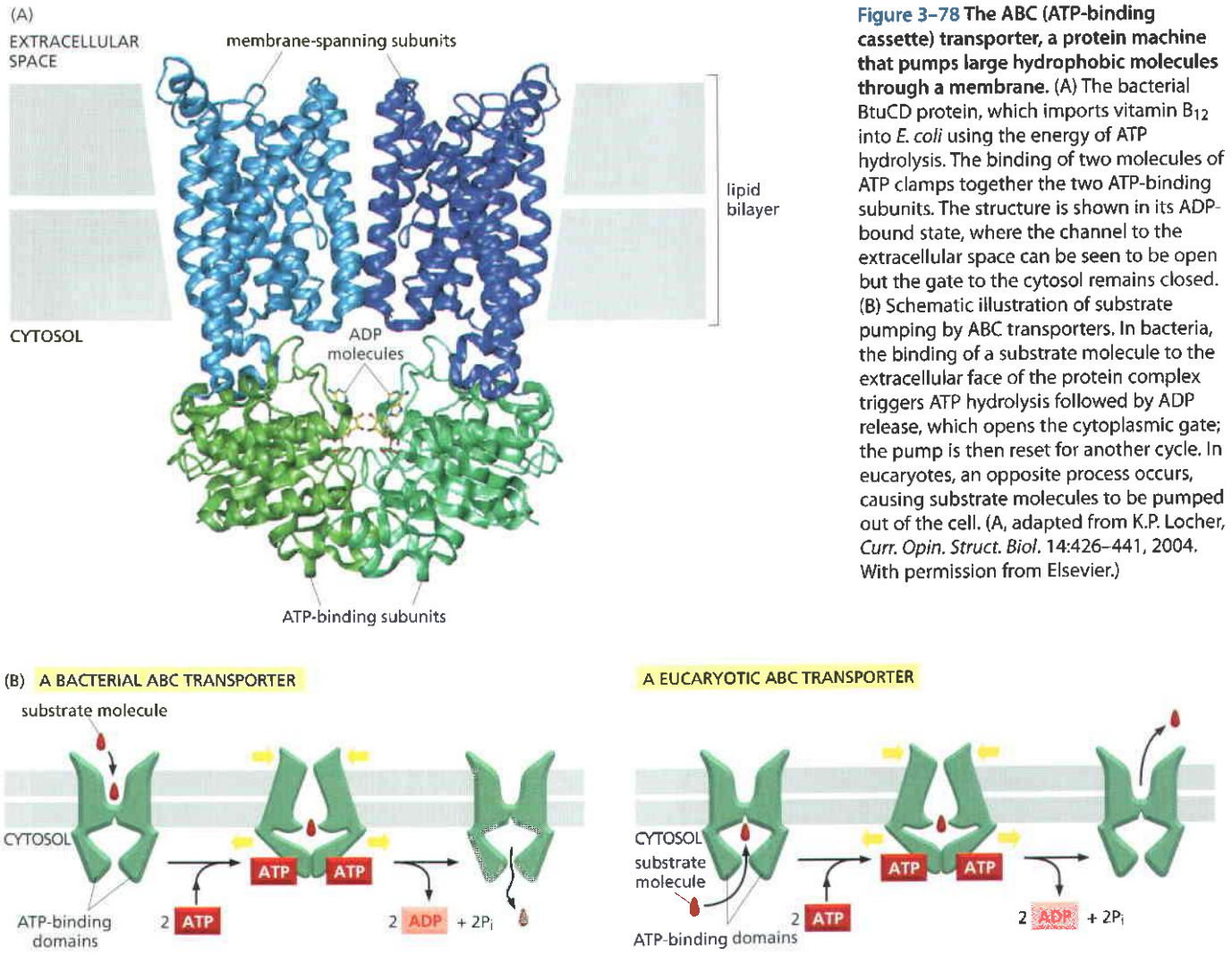


Figure 3–78 The ABC (ATP-binding cassette) transporter, a protein machine that pumps large hydrophobic molecules through a membrane. (A) The bacterial BtuCD protein, which imports vitamin B_{12} into *E. coli* using the energy of ATP hydrolysis. The binding of two molecules of ATP clamps together the two ATP-binding subunits. The structure is shown in its ADP-bound state, where the channel to the extracellular space can be seen to be open but the gate to the cytosol remains closed. (B) Schematic illustration of substrate pumping by ABC transporters. In bacteria, the binding of a substrate molecule to the extracellular face of the protein complex triggers ATP hydrolysis followed by ADP release, which opens the cytoplasmic gate; the pump is then reset for another cycle. In eucaryotes, an opposite process occurs, causing substrate molecules to be pumped out of the cell. (A, adapted from K.P. Locher, *Curr. Opin. Struct. Biol.* 14:426–441, 2004. With permission from Elsevier.)

serving to remove toxic molecules at the mucosal surface of the intestinal tract, for example, or at the blood–brain barrier. The study of ABC transporters is of intense interest in clinical medicine, because the overproduction of proteins in this class contributes to the resistance of tumor cells to chemotherapeutic drugs. And in bacteria, the same type of proteins primarily function to import essential nutrients into the cell.

The ABC transporter is a tetramer, with a pair of membrane-spanning subunits linked to a pair of ATP binding subunits located just below the plasma membrane (**Figure 3–78**A). As in other examples we have discussed, the hydrolysis of the bound ATP molecules drives conformational changes in the protein, transmitting forces that cause the membrane-spanning subunits to move their bound molecules across the lipid bilayer (Figure 3–78B).

Humans have invented many different types of mechanical pumps, and it should not be surprising that cells also contain membrane-bound pumps that function in other ways. Among the most notable are the rotary pumps that couple the hydrolysis of ATP to the transport of H^+ ions (protons). These pumps resemble miniature turbines, and they are used to acidify the interior of lysosomes and other eucaryotic organelles. Like other ion pumps that create ion gradients, they can function in reverse to catalyze the reaction ADP + P_i → ATP, if the gradient across their membrane of the ion that they transport is steep enough.

One such pump, the ATP synthase, harnesses a gradient of proton concentration produced by electron transport processes to produce most of the ATP used in the living world. This ubiquitous pump has a central role in energy conversion, and we shall discuss its three-dimensional structure and mechanism in Chapter 14.

Proteins Often Form Large Complexes That Function as Protein Machines <ACTT> <ATCG>

Large proteins formed from many domains are able to perform more elaborate functions than small, single-domain proteins. But large protein assemblies formed from many protein molecules perform the most impressive tasks. Now that it is possible to reconstruct most biological processes in cell-free systems in the laboratory, it is clear that each of the central processes in a cell—such as DNA replication, protein synthesis, vesicle budding, or transmembrane signaling—is catalyzed by a highly coordinated, linked set of 10 or more proteins. In most such *protein machines*, an energetically favorable reaction such as the hydrolysis of bound nucleoside triphosphates (ATP or GTP) drives an ordered series of conformational changes in one or more of the individual protein subunits, enabling the ensemble of proteins to move coordinately. In this way, each enzyme can be moved directly into position, as the machine catalyzes successive reactions in a series. This is what occurs, for example, in protein synthesis on a ribosome (discussed in Chapter 6)—or in DNA replication, where a large multiprotein complex moves rapidly along the DNA (discussed in Chapter 5).

Cells have evolved protein machines for the same reason that humans have invented mechanical and electronic machines. For accomplishing almost any task, manipulations that are spatially and temporally coordinated through linked processes are much more efficient than the use of individual tools.

Protein Machines with Interchangeable Parts Make Efficient Use of Genetic Information

To probe more deeply into the nature of protein machines, we shall consider a relatively simple one: the **SCF ubiquitin ligase**. This protein complex binds different "target proteins" at different times in the cell cycle, and it covalently adds multiubiquitin polypeptide chains to these proteins. Its C-shaped structure is formed from five protein subunits, the largest of which is a molecule that serves as a *scaffold protein* on which the rest of the structure is built. The structure underlies a remarkable mechanism (**Figure 3–79**). At one end of the C is an E2 ubiquitin-conjugating enzyme. At the other end is a substrate-binding arm, a subunit known as an *F-box protein*. These two subunits are separated by a gap of about 5 nm. When this protein complex is activated, the F-box protein binds to a specific site on a target protein, positioning the protein in the gap so that some of its lysine side chains contact the ubiquitin-conjugating enzyme. This enzyme can then catalyze the repeated addition of a ubiquitin polypeptide to these lysines (see Figure 3–79C), producing a polyubiquitin chain that marks the target protein for rapid destruction in a proteasome (see p. 393).

In this manner, specific proteins are targeted for rapid destruction in response to specific signals, thereby helping to drive the cell cycle (discussed in Chapter 17). The timing of the destruction often involves creating a specific pattern of phosphorylation on the target protein that is required for its recognition by the F-box subunit. It also requires the activation of an SCF ubiquitin ligase that carries the appropriate substrate-binding arm. Many of these arms (the F-box subunits) are interchangeable in the protein complex (see Figure 3–79B), and there are more than 70 human genes that encode them.

As emphasized previously, once a successful protein has evolved, its genetic information tends to be duplicated to produce a family of related proteins. Thus, for example, not only are there many F-box proteins—making possible the recognition of different sets of target proteins—but there is also a family of scaffolds (known as cullins) that give rise to a family of SCF-like ubiquitin ligases.

The pressure on organisms to minimize the number of genes (see p. 265) presumably helps to explain why RNA splicing is so prevalent in higher eucaryotes, allowing multiple related proteins to be synthesized from a single gene (discussed in Chapter 6). A protein machine like the SCF ubiquitin ligase, with its interchangeable parts, likewise makes economical use of the genetic information

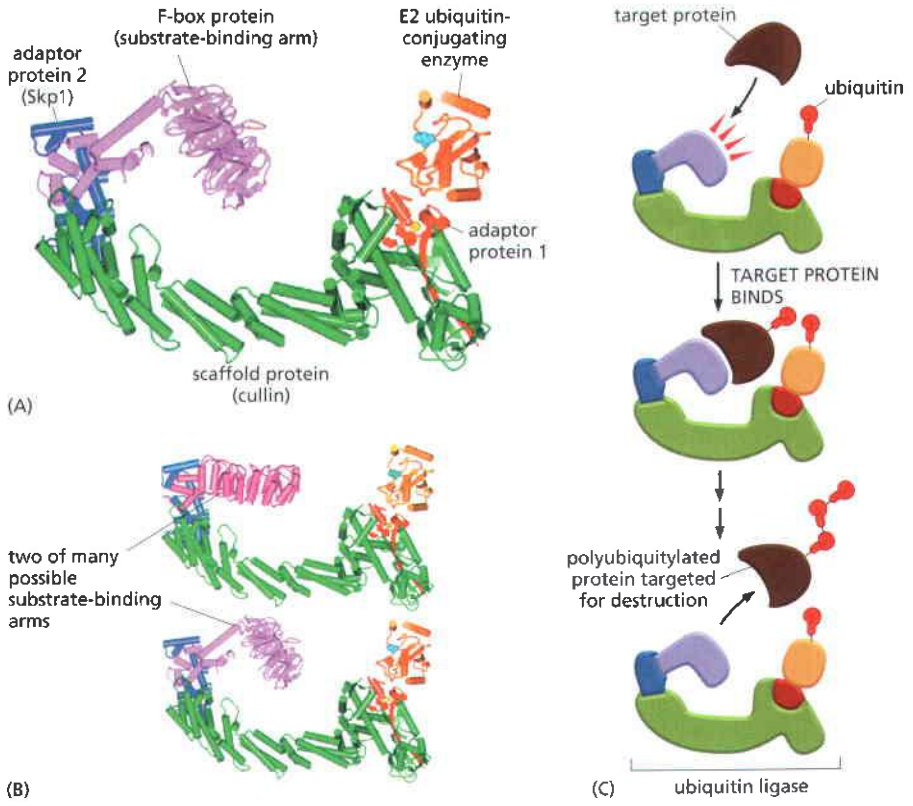


Figure 3–79 The structure and mode of action of a SCF ubiquitin ligase. (A) The structure of the five-protein complex that includes an E2 ubiquitin ligase. The protein denoted here as adapter protein 1 is the Rbx1/Hrt1 protein, adaptor protein 2 is the Skp1 protein, and the cullin is the Cul1 protein. (B) Comparison of the same complex with two different substrate-binding arms, the F-box proteins Skp2 *(top)* and β-trCP1 *(bottom)*, respectively. (C) The binding and ubiquitylation of a target protein by the SCF ubiquitin ligase. If, as indicated, a chain of ubiquitin molecules is added to the same lysine of the target protein, that protein is marked for rapid destruction by the proteasome. (A and B, adapted from G. Wu et al., *Mol. Cell* 11:1445–1456, 2003. With permission from Elsevier.)

in cells, inasmuch as new functions can evolve for the entire complex simply by producing an alternative version of one of its subunits.

The Activation of Protein Machines Often Involves Positioning Them at Specific Sites

As scientists have learned more of the details of cell biology, they have recognized increasing degrees of sophistication in cell chemistry. Thus, not only do we now know that protein machines play a predominant role, but it has recently become clear that most of these machines form at specific sites in the cell, being activated only where and when they are needed. Using fluorescent, GFP-tagged fusion proteins in living cells (see p. 593), cell biologists are able to follow the repositioning of individual proteins that occurs in response to specific signals. Thus, when certain extracellular signaling molecules bind to receptor proteins in the plasma membrane, they often recruit a set of other proteins to the inside surface of the plasma membrane to form protein machines that pass the signal on. As an example, Figure 3–80A illustrates the rapid movement of a protein kinase C (PKC) enzyme to a complex in the plasma membrane, where it associates with specific substrate proteins that it phosphorylates.

There are more than 10 distinct PKC enzymes in human cells, which differ both in their mode of regulation and in their functions. When activated, these enzymes move from the cytoplasm to different intracellular locations, forming specific complexes with other proteins that allow them to phosphorylate different protein substrates (Figure 3–80B). The SCF ubiquitin ligases can also move to specific sites of function at appropriate times. As will be explained when we discuss cell signaling in Chapter 15, the mechanisms frequently involve protein phosphorylation, as well as **scaffold proteins** that link together a set of activating, inhibiting, adaptor, and substrate proteins at a specific location in a cell.

This general phenomenon is known as *induced proximity*, and it explains the otherwise puzzling observation that slightly different forms of enzymes with the same catalytic site will often have very different biological functions. Cells change the locations of their proteins by covalently modifying them in a variety of different ways, as part of a "regulatory code" to be described next.

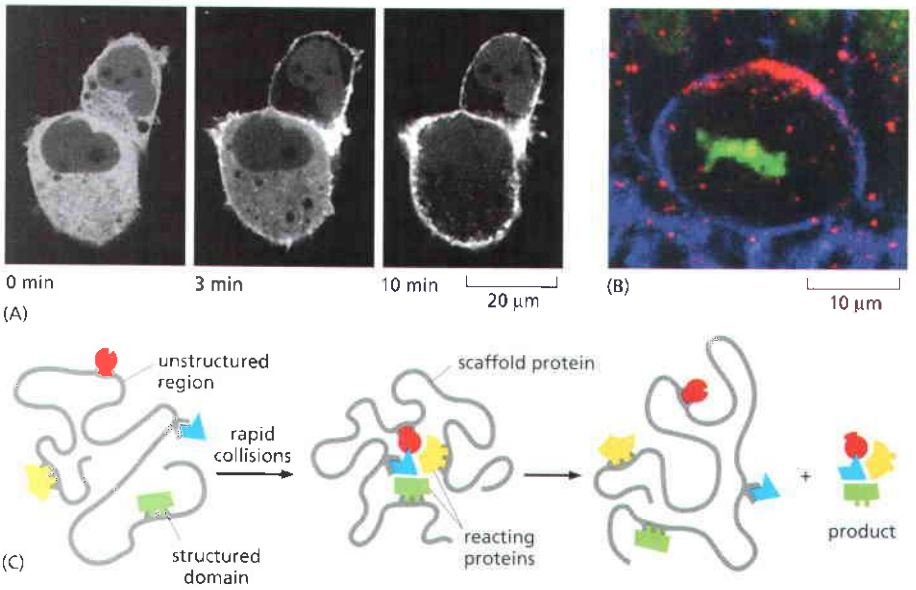


Figure 3–80 The assembly of protein machines at specific sites in a cell. (A) In response to a signal (here a phorbol ester), the gamma subspecies of protein kinase C moves rapidly from the cytosol to the plasma membrane. The protein kinase is fluorescent in these living cells because an engineered gene inside the cell encodes a fusion protein that links the kinase to green fluorescent protein (GFP). (B) The specific association of a different subspecies of protein kinase C (aPKC) with the apical tip of a differentiating neuroblast in an early *Drosophila* embryo. The kinase is stained *red*, and the cell nucleus *green*. (C) Diagram illustrating how a simple proximity created by scaffold proteins can greatly speed reactions in a cell. In this example, long unstructured regions of polypeptide chain in a large scaffold protein connect a series of structured domains that bind a set of reacting proteins. The unstructured regions serve as flexible "tethers" that greatly speed reaction rates by causing a rapid, random collision of all of the proteins that are bound to the scaffold. (For a simple example of tethering, see Figure 16–38.) (A, from N. Sakai et al, *J. Cell Biol.* 139:1465–1476, 1997. With permission from The Rockefeller University Press. B, courtesy of Andreas Wodarz, Institute of Genetics, University of Düsseldorf, Germany.)

These modifications create sites on proteins that bind them to particular scaffold proteins, thereby clustering the proteins required for particular reactions in specific regions of the cell. Most biological reactions are catalyzed by sets of 5 or more proteins, and such a clustering of proteins is often required for the reaction to occur. Scaffolds thereby allow cells to compartmentalize reactions even in the absence of membranes. Although only recently recognized as a widespread phenomenon, this type of clustering is particularly obvious in the cell nucleus (see Figure 4–69).

Many scaffolds appear to be quite different from the cullin illustrated previously in Figure 3–79: rather than holding their bound proteins in precise positions relative to each other, the interacting proteins are linked by unstructured regions of polypeptide chain. This tethers the proteins together, causing them to collide frequently with each other in random orientations—some of which will lead to a productive reaction (Figure 3–80C). In essence, this mechanism greatly speeds reactions by creating a very high local concentration of the reacting species. For this reason, the use of scaffold proteins represents an especially versatile way of controlling cell chemistry (see also Figure 15–61).

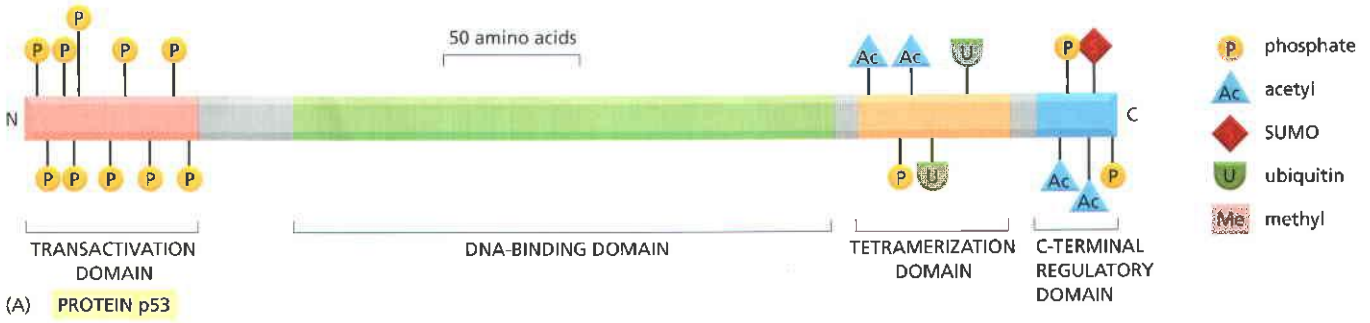
Many Proteins Are Controlled by Multisite Covalent Modification

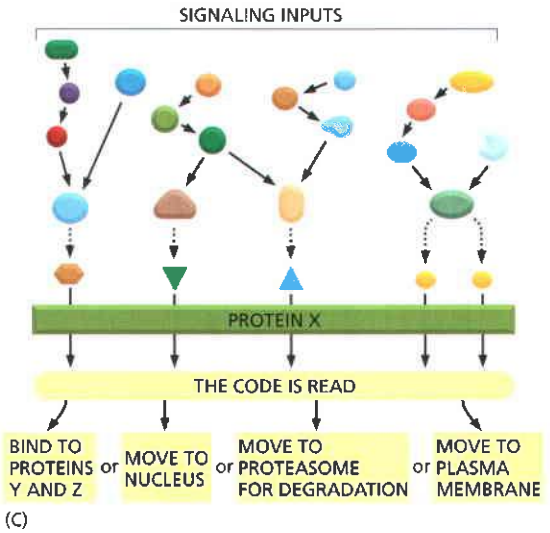
We have thus far described only one type of posttranslational modification of proteins—that in which a phosphate is covalently attached to an amino acid side chain (see Figure 3–64). But a large number of other such modifications also occur, more than 200 distinct types being known. To give a sense of the variety, Table 3–3 presents a subset of modifying groups with known regulatory roles. As

Table 3–3 Some Molecules Covalently Attached to Proteins Regulate Protein Function

MODIFYING GROUP	SOME PROMINENT FUNCTIONS
Phosphate on Ser, Thr, or Tyr	Drives the assembly of a protein into larger complexes (see Figure 15–19).
Methyl on Lys	Helps to creates histone code in chromatin through forming either mono-, di-, or tri-methyl lysine (see Figure 4–38).
Acetyl on Lys	Helps to creates histone code in chromatin (see Figure 4–38).
Palmityl group on Cys	This fatty acid addition drives protein association with membranes (see Figure 10–20).
***N*-acetylglucosamine** on Ser or Thr	Controls enzyme activity and gene expression in glucose homeostasis.
Ubiquitin on Lys	Monoubiquitin addition regulates the transport of membrane proteins in vesicles (see Figure 13–58).
	A polyubiquitin chain targets a protein for degradation (see Figure 3–79).

Ubiquitin is a 76 amino acid polypeptide; there are at least 10 other ubiquitin-related proteins, such as SUMO, that modify proteins in similar ways.





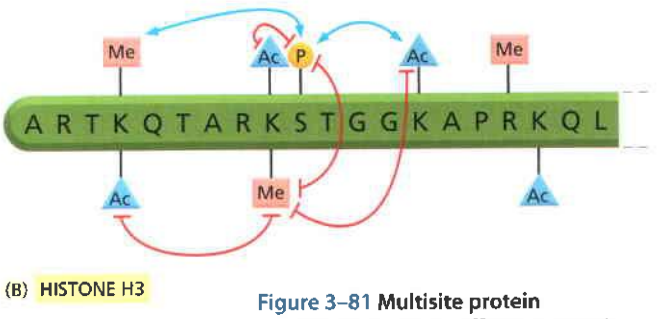


Figure 3–81 Multisite protein modification and its effects. A protein that carries a post-translational addition to more than one of its amino acid side chains can be considered to carry a combinatorial regulatory code. (A) The pattern of known covalent modifications to the protein p53; ubiquitin and SUMO are related polypeptides (see Table 3–3). (B) The possible modifications on the first 20 amino acids at the N-terminus of histone H3, showing not only their locations but also their activating *(blue)* and inhibiting *(red)* effects on the addition of neighboring covalent modifications. In addition to the effects shown, the acetylation and methylation of a lysine are mutually exclusive reactions (see Figure 4–38). (C) Diagram showing the general manner in which multisite modifications are added to (and removed from) a protein through signaling networks, and how the resulting combinatorial regulatory code on the protein is read to alter its behavior in the cell.

in phosphate addition, these groups are added and then removed from proteins according to the needs of the cell.

A large number of proteins are now known to be modified on more than one amino acid side chain, with different regulatory events producing a different pattern of such modifications. A striking example is the protein p53, which plays a central part in controlling a cell's response to adverse circumstances (see p. 1105). Through one of four different types of molecular additions, this protein can be modified at 20 different sites (**Figure 3–81**A). Because an enormous number of different combinations of these 20 modifications are possible, the protein's behavior can in principle be altered in a huge number of ways. Moreover, the pattern of modifications on a protein can determine its susceptibility to further modification, as illustrated by histone H3 in Figure 3–81B.

Cell biologists have only recently come to recognize that each protein's set of covalent modifications constitutes an important *combinatorial regulatory code.* As specific modifying groups are added to or removed from a protein, this code causes a different set of protein behaviors—changing the activity or stability of the protein, its binding partners, and its specific location within the cell (Figure 3–81C). This helps the cell respond rapidly and with great versatility to changes in its condition or environment.

A Complex Network of Protein Interactions Underlies Cell Function

There are many challenges facing cell biologists in this "post-genome" era when complete genome sequences are known. One is the need to dissect and reconstruct each one of the thousands of protein machines that exist in an organism such as ourselves. To understand these remarkable protein complexes, each must be reconstituted from its purified protein parts, so that we can study its detailed mode of operation under controlled conditions in a test tube, free from

all other cell components. This alone is a massive task. But we now know that each of these subcomponents of a cell also interacts with other sets of macromolecules, creating a large network of protein–protein and protein–nucleic acid interactions throughout the cell. To understand the cell, therefore, we need to analyze most of these other interactions as well.

We can gain some idea of the complexity of intracellular protein networks from a particularly well-studied example described in Chapter 16: the many dozens of proteins that interact with the actin cytoskeleton in the yeast *Saccharomyces cerevisiae* (see Figure 16–18). The extent of such protein–protein interactions can also be estimated more generally. An enormous amount of valuable information is now freely available in protein databases on the Internet: tens of thousands of three-dimensional protein structures plus tens of millions of protein sequences derived from the nucleotide sequences of genes. Scientists have been developing new methods for mining this great resource to increase our understanding of cells. In particular, computer-based bioinformatics tools are being combined with robotics and microarray technologies (see p. 574) to allow thousands of proteins to be investigated in a single set of experiments. **Proteomics** is a term that is often used to describe such research focused on the large-scale analysis of proteins, analogous to the term *genomics* describing the large-scale analysis of DNA sequences and genes.

Biologists use two different large-scale methods to map the direct binding interactions between the many different proteins in a cell. The initial method of choice was based on genetics: through an ingenious technique known as the yeast two-hybrid screen (see Figure 8–24), tens of thousands of interactions between thousands of proteins have been mapped in yeast, a nematode, and the fruit fly *Drosophila*. More recently, a biochemical method based on affinity tagging and mass spectroscopy has gained favor (discussed in Chapter 8), because it appears to produce fewer spurious results. The results of these and other analyses that predict protein binding interactions have been tabulated and organized in Internet databases. This allows a cell biologist studying a small set of proteins to readily discover which other proteins in the same cell are thought to bind to, and thus interact with, that set of proteins. When displayed graphically as a *protein interaction map*, each protein is represented by a box or dot in a two-dimensional network, with a straight line connecting those proteins that have been found to bind to each other.

When hundreds or thousands of proteins are displayed on the same map, the network diagram becomes bewilderingly complicated, serving to illustrate how much more we have to learn before we can claim to really understand the cell. Much more useful are small subsections of these maps, centered on a few proteins of interest. Thus, **Figure 3–82** shows a network of protein–protein interactions for the five proteins that form the SCF ubiquitin ligase in a yeast cell (see Figure 3–79). Four of the subunits of this ligase are located at the bottom right of Figure 3–82. The remaining subunit, the F-box protein that serves as its substrate-binding arm, appears as a set of 15 different gene products that bind to adaptor protein 2 (the Skp1 protein). Along the top and left of the figure are sets of additional protein interactions marked with *yellow* and *green* shading: as indicated, these protein sets function at the origin of DNA replication, in cell cycle regulation, in methionine synthesis, in the kinetochore, and in vacuolar H^+-ATPase assembly. We shall use this figure to explain how such protein interaction maps are used, and what they do and do not mean.

1. Protein interaction maps are useful for identifying the likely function of previously uncharacterized proteins. Examples are the products of the genes that have thus far only been inferred to exist from the yeast genome sequence, which are the six proteins in the figure that lack a simple three-letter abbreviation (*white letters* beginning with Y). One, the product of so-called *open reading frame* YDR196C, is located in the origin of replication group, and it is therefore likely to have a role in starting new replication forks. The remaining five in this diagram are F-box proteins that bind to Skp1; these are therefore likely to function as part of the ubiquitin ligase, serving as substrate-binding arms that recognize different target proteins.

However, as we discuss next, neither assignment can be considered certain without additional data.

2. Protein interaction networks need to be interpreted with caution because, as a result of evolution making efficient use of each organism's genetic information, the same protein can be used as part of two different protein complexes that have different types of functions. Thus, although protein A binds to protein B and protein B binds to protein C, proteins A and C need not function in the same process. For example, we know from detailed biochemical studies that the functions of Skp1 in the kinetochore and in vacuolar H^+-ATPase assembly *(yellow shading)* are separate from its function in the SCF ubiquitin ligase. In fact, only the remaining three functions of Skp1 illustrated in the diagram—methionine synthesis, cell cycle regulation, and origin of replication *(green shading)*—involve ubiquitylation.
3. In cross-species comparisons, those proteins displaying similar patterns of interactions in the two protein interaction maps are likely to have the same function in the cell. Thus, as scientists generate more and more highly detailed maps for multiple organisms, the results will become increasingly useful for inferring protein function. These map comparisons are a particularly powerful tool for deciphering the functions of human proteins. There is a vast amount of direct information about protein function that can be obtained from genetic engineering, mutational, and

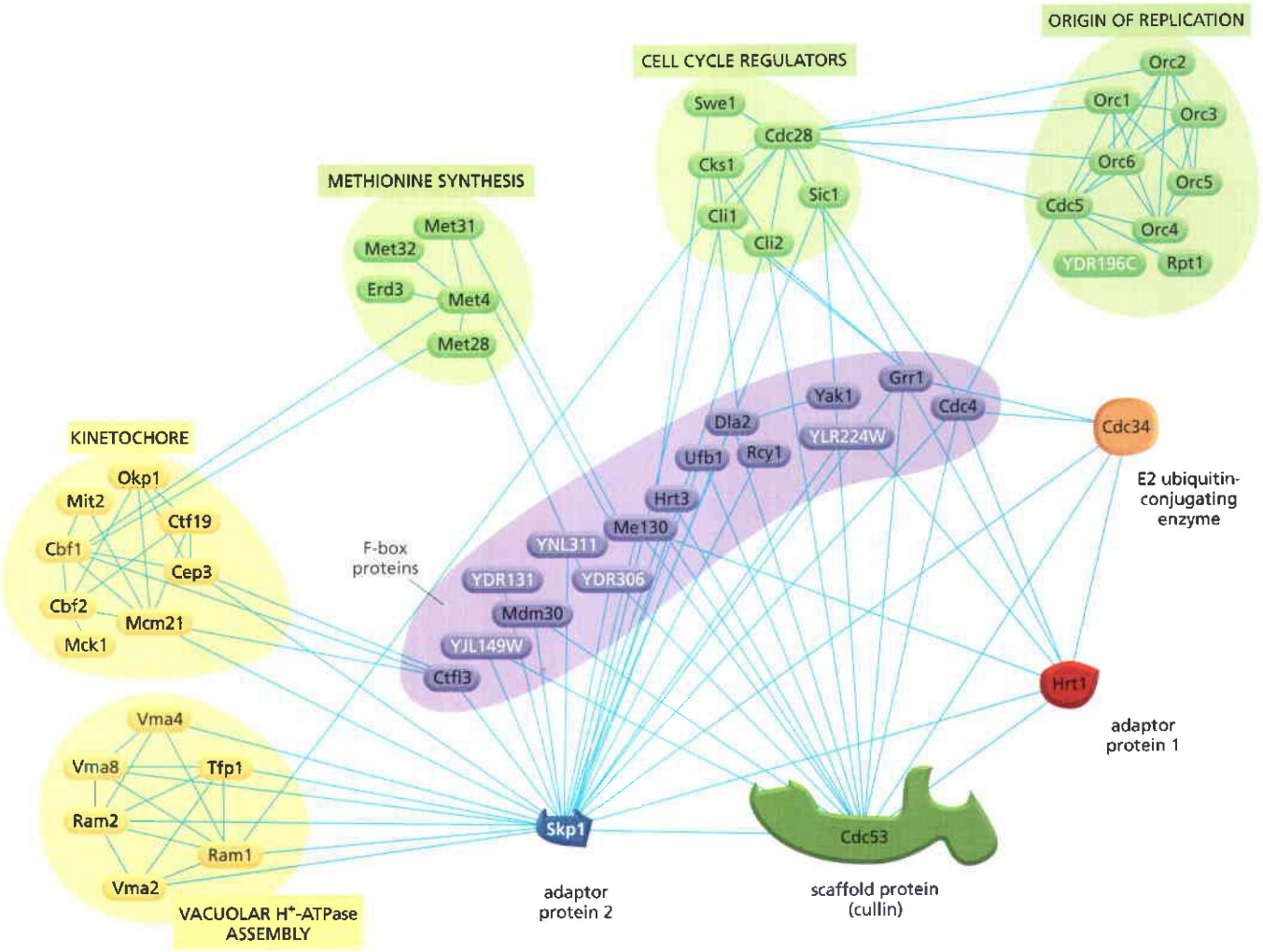


Figure 3–82 A map of some protein– protein interactions of the SCF ubiquitin ligase and other proteins in the yeast *S. cerevisiae*. The symbols and/or colors used for the 5 proteins of the ligase are those in Figure 3–79. Note that 15 different F-box proteins are shown (*purple*); those with *white* lettering (beginning with Y) are only known from the genome sequence as open reading frames. For additional details, see text. (Courtesy of Peter Bowers and David Eisenberg, UCLA-DOE Institute for Genomics and Proteomics, UCLA.)



Figure 3–83 A network of protein-binding interactions in a yeast cell. Each line connecting a pair of dots (proteins) indicates a protein–protein interaction. (From A. Guimerá and M. Sales-Pardo, *Mol. Syst. Biol.* 2:42, 2006. With permission from Macmillan Publishers Ltd.)

genetic analyses in model organisms—such as yeast, worms, and flies—that is not available in humans.

The available data suggest that a typical protein in a human cell may interact with between 5 and 15 different partners. Often, each of the different domains in a multidomain protein binds to a different set of partners; in fact, we can speculate that the unusually extensive multidomain structures observed for human proteins may have evolved to facilitate these interactions. Given the enormous complexity of the interacting networks of macromolecules in cells (Figure 3–83), deciphering their full functional meaning may well keep scientists busy for centuries.

Summary

Proteins can form enormously sophisticated chemical devices, whose functions largely depend on the detailed chemical properties of their surfaces. Binding sites for ligands are formed as surface cavities in which precisely positioned amino acid side chains are brought together by protein folding. In this way, normally unreactive amino acid side chains can be activated to make and break covalent bonds. Enzymes are catalytic proteins that greatly speed up reaction rates by binding the high-energy transition states for a specific reaction path; they also perform acid catalysis and base catalysis simultaneously. The rates of enzyme reactions are often so fast that they are limited only by diffusion; rates can be further increased if enzymes that act sequentially on a substrate are joined into a single multienzyme complex, or if the enzymes and their substrates are confined to the same compartment of the cell.

Proteins reversibly change their shape when ligands bind to their surface. The allosteric changes in protein conformation produced by one ligand affect the binding of a second ligand, and this linkage between two ligand-binding sites provides a crucial mechanism for regulating cell processes. Metabolic pathways, for example, are controlled by feedback regulation: some small molecules inhibit and other small molecules activate enzymes early in a pathway. Enzymes controlled in this way generally form symmetric assemblies, allowing cooperative conformational changes to create a steep response to changes in the concentrations of the ligands that regulate them.

The expenditure of chemical energy can drive unidirectional changes in protein shape. By coupling allosteric shape changes to ATP hydrolysis, for example, proteins can do useful work, such as generating a mechanical force or moving for long distances in a single direction. The three-dimensional structures of proteins, determined by x-ray crystallography, have revealed how a small local change caused by nucleoside triphosphate hydrolysis is amplified to create major changes elsewhere in the protein. By such means, these proteins can serve as input–output devices that transmit information, as assembly factors, as motors, or as membrane-bound pumps. Highly efficient protein machines are formed by incorporating many different protein molecules into larger assemblies that coordinate the allosteric movements of the individual components. Such machines are now known to perform many of the most important reactions in cells.

Proteins are subjected to many reversible post-translational modifications, such as the covalent addition of a phosphate or an acetyl group to a specific amino acid side chain. The addition of these modifying groups is used to regulate the activity of a protein, changing its conformation, its binding to other proteins and its location inside the cell. A typical protein in a cell will interact with more than five different partners. Using the new technologies of proteomics, biologists can analyze thousands of proteins in one set of experiments. One important result is the production of detailed protein interaction maps, which aim at describing all of the binding interactions between the thousands of distinct proteins in a cell.

PROBLEMS

Which statements are true? Explain why or why not.

3–1 Each strand in a β sheet is a helix with two amino acids per turn.

3–2 Loops of polypeptide that protrude from the surface of a protein often form the binding sites for other molecules.

3–3 An enzyme reaches a maximum rate at high substrate concentration because it has a fixed number of active sites where substrate binds.

3–4 Higher concentrations of enzyme give rise to a higher turnover number.

3–5 Enzymes such as aspartate transcarbamoylase that undergo cooperative allosteric transitions invariably contain multiple identical subunits.

3–6 Continual addition and removal of phosphates by protein kinases and protein phosphatases is wasteful of energy—since their combined action consumes ATP—but it is a necessary consequence of effective regulation by phosphorylation.

Discuss the following problems.

3–7 Consider the following statement. "To produce one molecule of each possible kind of polypeptide chain, 300 amino acids in length, would require more atoms than exist in the universe." Given the size of the universe, do you suppose this statement could possibly be correct? Since counting atoms is a tricky business, consider the problem from the standpoint of mass. The mass of the observable universe is estimated to be about 10^{80} grams, give or take an order of magnitude or so. Assuming that the average mass of an amino acid is 110 daltons, what would be the mass of one molecule of each possible kind of polypeptide chain 300 amino acids in length? Is this greater than the mass of the universe?

3–8 A common strategy for identifying distantly related proteins is to search the database using a short signature sequence indicative of the particular protein function. Why is it better to search with a short sequence than with a long sequence? Do you not have more chances for a 'hit' in the database with a long sequence?

3–9 The so-called kelch motif consists of a four-stranded β sheet, which forms what is known as a β propeller. It is usually found to be repeated four to seven times, forming a kelch repeat domain in a multidomain protein. One such kelch repeat domain is shown in **Figure Q3–1**. Would you classify this domain as an 'in-line' or 'plug-in' type domain?

3–10 Titin, which has a molecular weight of 3×10^6 daltons, is the largest polypeptide yet described. Titin molecules extend from muscle thick filaments to the Z disc; they are thought to act as springs to keep the thick filaments centered in the sarcomere. Titin is composed of a large number of repeated immunoglobulin (Ig) sequences of 89 amino acids, each of which is folded into a domain about 4 nm in length (**Figure Q3–2A**).

You suspect that the springlike behavior of titin is caused by the sequential unfolding (and refolding) of individual Ig domains. You test this hypothesis using the atomic force microscope, which allows you to pick up one end of a protein molecule and pull with an accurately measured force. For a fragment of titin containing seven repeats of the Ig domain, this experiment gives the sawtooth force-versus-extension curve shown in Figure Q3–2B. When the experiment is repeated in a solution of 8 M urea (a protein denaturant), the peaks disappear and the measured extension becomes much longer for a given force. If the experiment is repeated after the protein has been cross-linked by treatment with glutaraldehyde, once again the peaks disappear but the extension becomes much smaller for a given force.

A. Are the data consistent with your hypothesis that titin's springlike behavior is due to the sequential unfolding of individual Ig domains? Explain your reasoning.

B. Is the extension for each putative domain-unfolding event the magnitude you would expect? (In an extended polypeptide chain, amino acids are spaced at intervals of 0.34 nm.)

C. Why is each successive peak in Figure Q3–2B a little higher than the one before?

D. Why does the force collapse so abruptly after each peak?

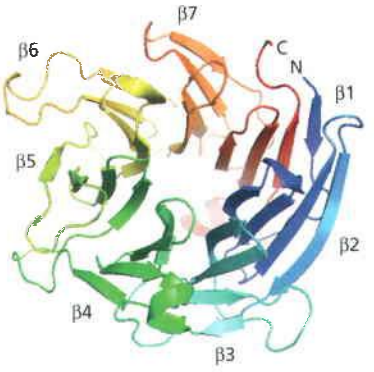


Figure Q3–1 The kelch repeat domain of galactose oxidase from *D. dendroides* (Problem 3–9). The seven individual β propellers are indicated. The N- and C-termini are indicated by N and C.

3–11 It is often said that protein complexes are made from subunits (that is, individually synthesized proteins) rather than as one long protein because the former is more likely to give a correct final structure.

A. Assuming that the protein synthesis machinery incorporates one incorrect amino acid for each 10,000 it inserts,

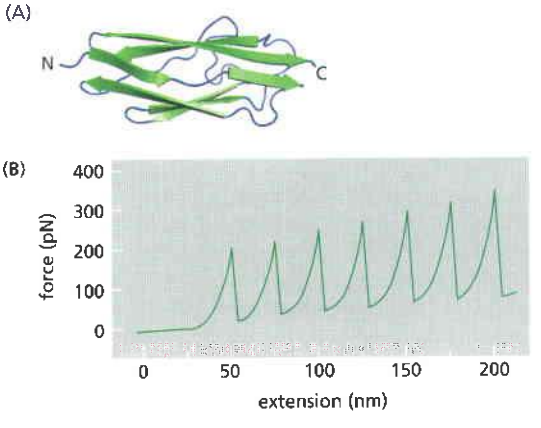


Figure Q3–2 Springlike behavior of titin (Problem 3–10). (A) The structure of an individual Ig domain. (B) Force in piconewtons versus extension in nanometers obtained by atomic force microscopy.

calculate the fraction of bacterial ribosomes that would be assembled correctly if the proteins were synthesized as one large protein versus built from individual proteins? For the sake of calculation assume that the ribosome is composed of 50 proteins, each 200 amino acids in length, and that the subunits—correct and incorrect—are assembled with equal likelihood into the complete ribosome. [The probability that a polypeptide will be made correctly, P_C, equals the fraction correct for each operation, f_C, raised to a power equal to the number of operations, n: $P_C = (f_C)^n$. For an error rate of 1/10,000, $f_C = 0.9999$.]

B. Is the assumption that correct and incorrect subunits assemble equally well likely to be true? Why or why not? How would a change in that assumption affect the calculation in part A?

3–12 Rous sarcoma virus (RSV) carries an oncogene called *Src*, which encodes a continuously active protein tyrosine kinase that leads to unchecked cell proliferation. Normally, Src carries an attached fatty acid (myristoylate) group that allows it to bind to the cytoplasmic side of the plasma membrane. A mutant version of Src that does not allow attachment of myristoylate does not bind to the membrane. Infection of cells with RSV encoding either the normal or the mutant form of Src leads to the same high level of protein tyrosine kinase activity, but the mutant Src does not cause cell proliferation.

A. Assuming that the normal Src is all bound to the plasma membrane and that the mutant Src is distributed throughout the cytoplasm, calculate their relative concentrations in the neighborhood of the plasma membrane. For the purposes of this calculation, assume that the cell is a sphere with a radius of 10 μm and that the mutant Src is distributed throughout, whereas the normal Src is confined to a 4-nm-thick layer immediately beneath the membrane. [For this problem, assume that the membrane has no thickness. The volume of a sphere is $(4/3)\pi r^3$.]

B. The target (X) for phosphorylation by *Src* resides in the membrane. Explain why the mutant Src does not cause cell proliferation.

3–13 An antibody binds to another protein with an equilibrium constant, K, of 5×10^9 M^{-1}. When it binds to a second, related protein, it forms three fewer hydrogen bonds, reducing its binding affinity by 2.8 kcal/mole. What is the K for its binding to the second protein? (Free-energy change is related to the equilibrium constant by the equation $\Delta G^\circ = -2.3\ RT \log K$, where R is 1.98×10^{-3} kcal/(mole K) and T is 310 K.)

3–14 The protein SmpB binds to a special species of tRNA, tmRNA, to eliminate the incomplete proteins made from truncated mRNAs in bacteria. If the binding of SmpB to tmRNA is plotted as fraction tmRNA bound versus SmpB concentration, one obtains a symmetrical S-shaped curve as shown in **Figure Q3–3**. This curve is a visual display of a very useful relationship between K_d and concentration, which has broad applicability. The general expression for fraction of ligand bound is derived from the equation for K_d (K_d = [Pr][L]/[Pr–L]) by substituting ($[L]_{TOT} - [L]$) for [Pr–L] and rearranging. Because the total concentration of ligand ($[L]_{TOT}$) is equal to the free ligand ([L]) plus bound ligand ([Pr–L]),

$$\text{fraction bound} = [L]/[L]_{TOT} = [Pr]/([Pr] + K_d)$$

For SmpB and tmRNA, the fraction bound = $[tmRNA]/[tmRNA]_{TOT} = [SmpB]/([SmpB] + K_d)$. Using this relationship, calculate the fraction of tmRNA bound for SmpB concentrations equal to 10^4K_d, 10^3K_d, 10^2K_d, 10^1K_d, K_d, $10^{-1}K_d$, $10^{-2}K_d$, $10^{-3}K_d$, and $10^{-4}K_d$.

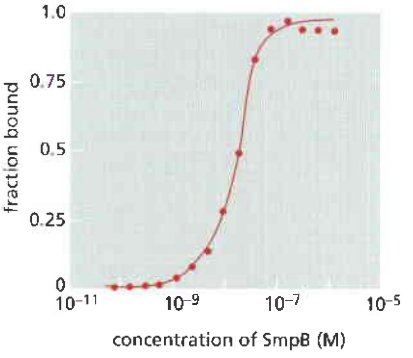


Figure Q3–3 Fraction of tmRNA bound versus SmpB concentration (Problem 3–14).

3–15 Many enzymes obey simple Michaelis–Menten kinetics, which are summarized by the equation

$$\text{rate} = V_{max}[S]/([S] + K_m)$$

where V_{max} = maximum velocity, [S] = concentration of substrate, and K_m = the Michaelis constant.

It is instructive to plug a few values of [S] into the equation to see how rate is affected. What are the rates for [S] equal to zero, equal to K_m, and equal to infinite concentration?

3–16 The enzyme hexokinase adds a phosphate to D-glucose but ignores its mirror image, L-glucose. Suppose that you were able to synthesize hexokinase entirely from D-amino acids, which are the mirror image of the normal L-amino acids.

A. Assuming that the 'D' enzyme would fold to a stable conformation, what relationship would you expect it to bear to the normal 'L' enzyme?

B. Do you suppose the 'D' enzyme would add a phosphate to L-glucose, and ignore D-glucose?

3–17 How do you suppose that a molecule of hemoglobin is able to bind oxygen efficiently in the lungs, and yet release it efficiently in the tissues?

3–18 Synthesis of the purine nucleotides AMP and GMP proceeds by a branched pathway starting with ribose 5-phosphate (R5P), as shown schematically in **Figure Q3–4**. Using the principles of feedback inhibition, propose a regulatory strategy for this pathway that ensures an adequate supply of both AMP and GMP and minimizes the buildup of the intermediates (*A–I*) when supplies of AMP and GMP are adequate.

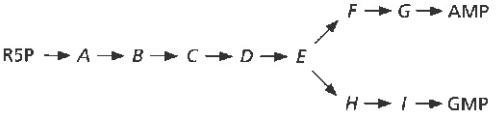


Figure Q3–4 Schematic diagram of the metabolic pathway for synthesis of AMP and GMP from R5P (Problem 3–18).

REFERENCES

General

Berg JM, Tymoczko JL & Stryer L (2006) Biochemistry, 6th ed. New York: WH Freeman.

Branden C & Tooze J (1999) Introduction to Protein Structure, 2nd ed. New York: Garland Science.

Dickerson, RE (2005) Present at the Flood: How Structural Molecular Biology Came About. Sunderland, MA: Sinauer

Kyte J (2006) Structure in Protein Chemistry. New York: Routledge.

Petsko GA & Ringe D (2004) Protein Structure and Function. London: New Science Press.

Perutz M (1992) Protein Structure: New Approaches to Disease and Therapy. New York: WH Freeman.

The Shape and Structure of Proteins

Anfinsen CB (1973) Principles that govern the folding of protein chains. *Science* 181:223–230.

Bray D (2005) Flexible peptides and cytoplasmic gels. *Genome Biol* 6:106–109.

Burkhard P, Stetefeld J & Strelkov SV (2001) Coiled coils: a highly versatile protein folding motif. *Trends Cell Biol* 11:82–88.

Caspar DLD & Klug A (1962) Physical principles in the construction of regular viruses. *Cold Spring Harb Symp Quant Biol* 27:1–24.

Doolittle RF (1995) The multiplicity of domains in proteins. *Annu Rev Biochem* 64:287–314.

Eisenberg D (2003) The discovery of the alpha-helix and beta-sheet, the principle structural features of proteins. *Proc Natl Acad Sci USA* 100:11207–11210.

Fraenkel-Conrat H & Williams RC (1955) Reconstitution of active tobacco mosaic virus from its inactive protein and nucleic acid components. *Proc Natl Acad Sci USA* 41:690–698.

Goodsell DS & Olson AJ (2000) Structural symmetry and protein function. *Annu Rev Biophys Biomol Struct* 29:105–153.

Harrison SC (1992) Viruses. *Curr Opin Struct Biol* 2:293–299.

Harrison SC (2004) Whither structural biology? *Nature Struct Mol Biol* 11:12–15.

Hudder A, Nathanson L & Deutscher MP (2003) Organization of mammalian cytoplasm. *Mol Cell Biol* 23:9318–9326.

International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. *Nature* 409:860–921.

Meiler J & Baker D (2003) Coupled prediction of protein secondary and tertiary structure. *Proc Natl Acad Sci USA* 100:12105–12110.

Nomura M (1973) Assembly of bacterial ribosomes. *Science* 179: 864–873.

Orengo CA & Thornton JM (2005) Protein families and their evolution—a structural perspective. *Annu Rev Biochem* 74:867–900.

Pauling L & Corey RB (1951) Configurations of polypeptide chains with favored orientations around single bonds: two new pleated sheets. *Proc Natl Acad Sci USA* 37:729–740.

Pauling L, Corey RB & Branson HR (1951) The structure of proteins: two hydrogen-bonded helical configurations of the polypeptide chain. *Proc Natl Acad Sci USA* 37:205–211.

Ponting CP, Schultz J, Copley RR et al. (2000) Evolution of domain families. *Adv Protein Chem* 54:185–244.

Trinick J (1992) Understanding the functions of titin and nebulin. *FEBS Lett* 307:44–48.

Vogel C, Bashton M, Kerrison ND et al (2004) Structure, function and evolution of multidomain proteins. *Curr Opin Struct Biol* 14:208–216.

Zhang C & Kim SH (2003) Overview of structural genomics: from structure to function. *Curr Opin Chem Biol* 7:28–32.

Protein Function

Alberts B (1998) The cell as a collection of protein machines: preparing the next generation of molecular biologists. *Cell* 92:291–294.

Benkovic SJ (1992) Catalytic antibodies. *Annu Rev Biochem* 61:29–54.

Berg OG & von Hippel PH (1985) Diffusion-controlled macromolecular interactions. *Annu Rev Biophys Biophys Chem* 14:131–160.

Bhattacharyya RP, Remenyi A, Yeh BJ & Lim WA (2006) Domains, motifs, and scaffolds: The role of modular interactions in the evolution and wiring of cell signaling circuits. *Annu Rev Biochem* 75:655–680.

Bourne HR (1995) GTPases: a family of molecular switches and clocks. *Philos Trans R Soc Lond B* 349:283–289.

Braden BC & Poljak RJ (1995) Structural features of the reactions between antibodies and protein antigens. *FASEB J* 9:9–16.

Dickerson RE & Geis I (1983) Hemoglobin: Structure, Function, Evolution and Pathology. Menlo Park, CA: Benjamin Cummings.

Dressler D & Potter H (1991) Discovering Enzymes. New York: Scientific American Library.

Eisenberg D, Marcotte, EM, Xenarios, I & Yeates TO (2000) Protein function in the post-genomic era. *Nature* 405:823–826.

Fersht AR (1999) Structure and Mechanisms in Protein Science: A Guide to Enzyme Catalysis. New York: WH Freeman.

Johnson, LN & Lewis RJ (2001) Structural basis for control by phosphorylation. *Chem Rev* 101:2209–2242.

Kantrowitz ER & Lipscomb WN (1988) *Escherichia coli* aspartate transcarbamoylase: the relation between structure and function. *Science* 241:669–674.

Khosla C & Harbury PB (2001) Modular enzymes. *Nature* 409:247–252.

Kim E & Sheng M (2004) PDZ domain proteins of synapses. *Nature Rev Neurosci* 5:771–781.

Koshland DE, Jr (1984) Control of enzyme activity and metabolic pathways. *Trends Biochem Sci* 9:155–159.

Kraut DA, Carroll KS & Herschlag D (2003) Challenges in enzyme mechanism and energetics. *Annu Rev Biochem* 72:517–571.

Krogan NJ, Cagney G, Yu H et al (2006) Global landscape of protein complexes in the yeast *Saccharomyces cerevisisae*. *Nature* 440:637–643.

Lichtarge O, Bourne HR & Cohen FE (1996) An evolutionary trace method defines binding surfaces common to protein families. *J Mol Biol* 257:342–358.

Marcotte EM, Pellegrini M, Ng HL et al (1999) Detecting protein function and protein–protein interactions from genome sequences. *Science* 285:751–753.

Monod J, Changeux JP & Jacob F (1963) Allosteric proteins and cellular control systems. *J Mol Biol* 6:306–329.

Pawson T & Nash P (2003) Assembly of regulatory systems through protein interaction domains. *Science* 300:445–452.

Pavletich NP (1999) Mechanisms of cyclin-dependent kinase regulation: structures of Cdks, their cyclin activators, and Cip and INK4 inhibitors. *J Mol Biol* 287:821–828.

Pellicena P & Kuriyan J (2006) Protein-protein interactions in the allosteric regulation of protein kinases. *Curr Opin Struct Biol* 16:702–709.

Perutz M (1990) Mechanisms of Cooperativity and Allosteric Regulation in Proteins. Cambridge: Cambridge University Press.

Raushel F, Thoden, JB & Holden HM (2003) Enzymes with molecular tunnels. *Acc Chem Res* 36:539–548.

Radzicka A & Wolfenden R (1995) A proficient enzyme. *Science* 267:90–93.

Sato TK, Overduin M & Emr S (2001) Location, location, location: Membrane targeting directed by PX domains. *Science* 294:1881–1885.

Schramm VL (1998) Enzymatic transition states and transition state analog design. *Annu Rev Biochem* 67:693–720.

Schultz PG & Lerner RA (1995) From molecular diversity to catalysis: lessons from the immune system. *Science* 269:1835–1842.

Vale RD & Milligan RA (2000) The way things move: looking under the hood of molecular motor proteins. *Science* 288:88–95.

Vocadlo DJ, Davies GJ, Laine R & Withers SG (2001) Catalysis by hen egg-white lysozyme proceeds via a covalent intermediate. *Nature* 412:835–838.

Walsh C (2001) Enabling the chemistry of life. *Nature* 409:226–231.

Yang XJ (2005) Multisite protein modification and intramolecular signaling. *Oncogene* 24:1653–1662.

Zhu H, Bilgin M & Snyder M (2003) Proteomics. *Annu Rev Biochem* 72:783–812.